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OBSERVATIONS ON KERATIN DIGESTION BY MICROSPORUM GYPSEUM[1]

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(WITH 2 FIGURES)

Since the dermatophytes occur in superficial tissues (hair, skin, nails) of mammals, and since the chief proteinaceous constituent of these tissues is keratin, it has often been assumed that the dermatophytes are capable of digesting keratin. This presumed ability is of interest, because under normal conditions, keratins are resistant to hydrolysis by such proteolytic enzymes as pepsin or trypsin. The reason for the indigestibility of keratins must lie in their properties. These scleroproteins, as is well known, are insoluble in water, dilute acids and bases, ethanol, or salt solutions. They are highly polymerized compounds made up of polypeptide chains bound laterally by disulfide linkages which maintain the three-dimensional shape of the molecule.

Dermatophytes have been grown in culture with various types of keratin as substrata, but in no case have the cultures been observed in sufficient detail to establish that keratin is actually digested. For example, wool (Bonar and Dreyer, **1932**; Rogers,

[1] Portion of a thesis submitted to the faculty of Harvard University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The author wishes to express his gratitude to Prof. Wm. H. Weston for his encouragement and guidance during the course of this study and preparation of the manuscript. This investigation was carried out during the tenure of a Junior Research Fellowship, National Institute of Health.

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Hirschmann, and Humfeld, **1940**), human hair (Roberts, **1894**; Bonar and Dreyer, **1932**; Williams, **1934**, **1934a**, **1935**), and feathers (Macfadyen, **1894**; Tate, **1929**) have supported the growth of a variety of fungi belonging to this group. Growth on substrata as complex as hair, feathers, or wool cannot, however, be considered proof of the ability of these fungi to digest keratin.

A detailed study of the growth of dermatophytes on keratin-containing substrata was of interest, therefore, to establish the ability of these fungi to digest keratin, to consider the process with respect to general problems of the relation of organisms to insoluble substrata, and to compare the features of the growth *in vitro* with those encountered in the clinic. While these objectives of this investigation were not fully attained, such progress as has been made is presented in the hope that it may be of service to others working in the same field.

MATERIALS AND METHODS

Cows' horn, fingernails, wool, and human hair were selected as representative types of keratinized tissue and were prepared for study in the following ways. Cows' horn, obtained from a slaughter house, was reduced to a finely particulate state in two ways. In some cases a file was used, and, though tedious, this method was advantageous in that the particles were thin ribbons with a large surface area. In other cases, the horn was broken up and ground in a laboratory model Wiley mill to pass a 40 mesh screen. Both methods were designed to avoid the alteration of the properties of keratin which occurs when it is subjected to prolonged grinding in a ball mill (Routh, **1940**). For some observations the ground horn was incorporated in 2.0% tap water agar and sterilized by autoclaving at 15 lbs. pressure for 15 minutes. In a second series of observations heat sterilization was avoided by the use of chloroform, and the horn was added to autoclaved 2.0% agar which had cooled almost to the point of gelation. The medium was agitated while it was poured into Petri dishes, so that the particles were distributed and partially suspended in the agar. The fingernails were prepared by grinding in the Wiley mill, after which they were washed with 0.1% sodium taurocholate and fur-

ther cleaned by extraction with ether and acetone. The powder was added to 2.0% agar and sterilized by autoclaving. The wool, which was unscoured and solvent-extracted, was combed to remove debris, washed with soap and water, extracted repeatedly with ether, and dried. Following sterilization with 70% ethanol, it was placed in a sterile Petri dish and warm agar was added. The pigment in human hair obscured details of the fungus attack; therefore, grey hairs which were devoid of pigment were selected for study. The hair was prepared by washing it with 0.1% sodium taurocholate and rinsing with water and acetone. It was then extracted repeatedly with small portions of ether to remove any fats remaining and sterilized by immersing it in 70% ethanol for one hour. After sterilization a few strands were placed in a sterile Petri dish and warm agar was added.

The media were inoculated, and the cultures were incubated at 30° C. The cultures were observed frequently through the bottom of the inverted Petri dish with the 16 mm. objective of the microscope. Typical areas of the medium were selected, and photomicrographs of these areas were taken at suitable intervals by means of a Leitz Ibso attachment.

In addition to the observations on human hair embedded in agar, details of the attack on human hair were studied by placing hairs in Petri dishes with sterile tap water and inoculating with *Microsporum gypseum*. Hairs were removed from the cultures at various intervals after inoculation, mounted in lactophenol with acid fuchsin, and observed with the microscope.

OBSERVATIONS

Horn. In preliminary experiments several dermatophytes were grown on the horn medium. Plates of autoclaved horn medium were inoculated with three isolates each of *Microsporum gypseum, M. canis, M. Audouini, Trichophyton mentagrophytes, T. rubrum, T. tonsurans,* and *Epidermophyton floccosum.*[2] All of the fungi grew on the horn medium, but growth was diffuse and prostrate

[2] For these cultures, the author is indebted to Miss Margarita Silva of the School of Tropical Medicine, San Juan, Puerto Rico and to Dr. C. W. Emmons of the National Institute of Health, Bethesda, Md.

when compared with that on Sabouraud's agar. After two weeks, clear zones were formed under the centers of the colonies because of the partial disappearance of the horn particles from the medium. As a quantitative measure of the ability of the fungi to digest horn, the diameter of the clear zones did not prove satisfactory, because the margins of the zones were difficult to delimit, and the degree of transparency of the zones varied with the different strains. *M. gypseum*, *M. canis*, and *T. rubrum* produced zones which were larger with respect to the diameter of the colony and more transparent than the zones produced by the other fungi. *M. Audouini*, on the other hand, formed relatively small zones in which the particles of horn were translucent rather than transparent. The size and transparency of the cleared zones produced by one isolate of *M. gypseum* (S–159) suggested that it was the most active of the fungi tested in its ability to digest horn. This strain also grew rapidly and vigorously, and was therefore selected for use in the observations which follow.

In a series of detailed experiments with *Microsporum gypseum* (Bodin) Guiart and Grigorakis microscopic observations on individual fragments of horn showed that they became translucent and ultimately disappeared almost completely. A series of photomicrographs showing the digestion of a fragment of horn which had been ground in the Wiley mill and partially sterilized with chloroform is shown (FIG. 1). Although the chloroform did not effect complete sterilization of the horn, no contaminants appeared in the field selected for study. Since the fragment was located at some distance from the site of inoculation, the hyphae did not reach the vicinity of the particle until between 24 and 48 hours after the plate had been inoculated. The first hyphae to enter the field of observation were hyphae which radiated from the site of inoculation, and the direction of their growth appeared to be little influenced by the presence of the horn particles. After these hyphae had grown past a particle, they gave rise to numerous side branches which grew toward the particle and became closely applied to it (FIG. 1, b). Very little change in the appearance of the particle was apparent at this stage. By 96 hours after inoculation of the plate, however, a definite alteration of the appearance of the particle was visible (FIG. 1, c). The margin of the particle had

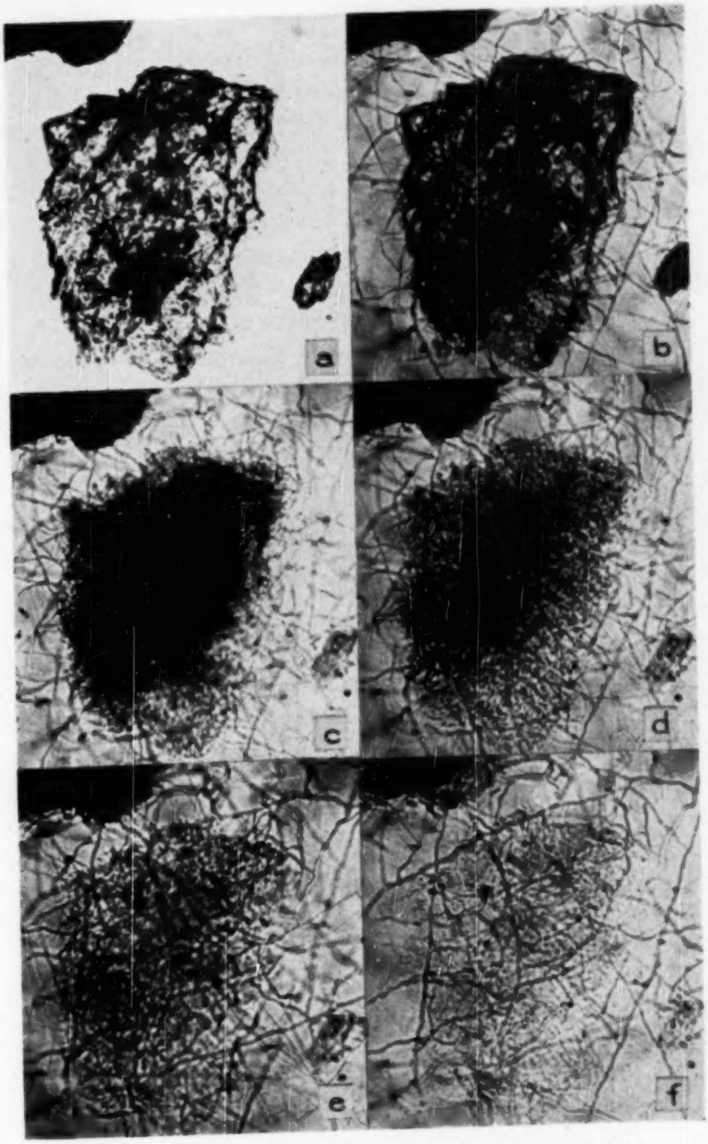

FIG. 1. Digestion of cows' horn by *Microsporum gypseum*.

become indistinct in outline and somewhat translucent. The zone of translucence gradually extended until the particle had all but disappeared at the end of 240 hours after inoculation.

While the sequence of events described in the preceding paragraph is typical, the time required for the digestion of a fragment varied considerably depending on its size, shape, proximity to the point of inoculation, and previous treatment. The thin ribbons of horn produced by filing and sterilized by autoclaving disappeared more rapidly than the thicker particles produced by the Wiley mill and sterilized with chloroform.

Fingernails. Preliminary observations on the digestion of fragments of human fingernails indicated that the process was very similar to the digestion of horn. The particles of fingernail in microscopic appearance closely resembled those of horn, they were approached by the hyphae in the same way, and they were digested in approximately the same length of time.

Wool. Wool represents a more complex form of keratinized tissue than does horn or fingernails, because the molecular units are oriented longitudinally, and each fiber is differentiated into a central region, the cortex, composed of spindle-shaped cells and an external sheath, the cuticle, made up of thin, overlapping scales. The digestion of wool was not studied in detail, because such studies are currently in progress in another laboratory; however, a brief description is included for the sake of completeness.

In one series, observations on the digestion of a wool fiber were made daily over a 10-day period. The fungus appeared to be able to penetrate the cuticle readily, since many of the fibers were attacked at several points along their length. Whether the hyphae gained entry to the cortex through or between the scales of the cuticle was not determined, but in any case, there was little distortion of the fiber at the point of penetration. After penetrating the cuticle, the hyphae grew longitudinally within the cortex. Accompanying the longitudinal growth were opaque areas which appeared as dark shadows. These opaque areas extended, and in some cases the longitudinally oriented hyphae could be distinguished within them. The substance of the cortex gradually disappeared, so that by a late stage little remained of the fiber but the cuticle which

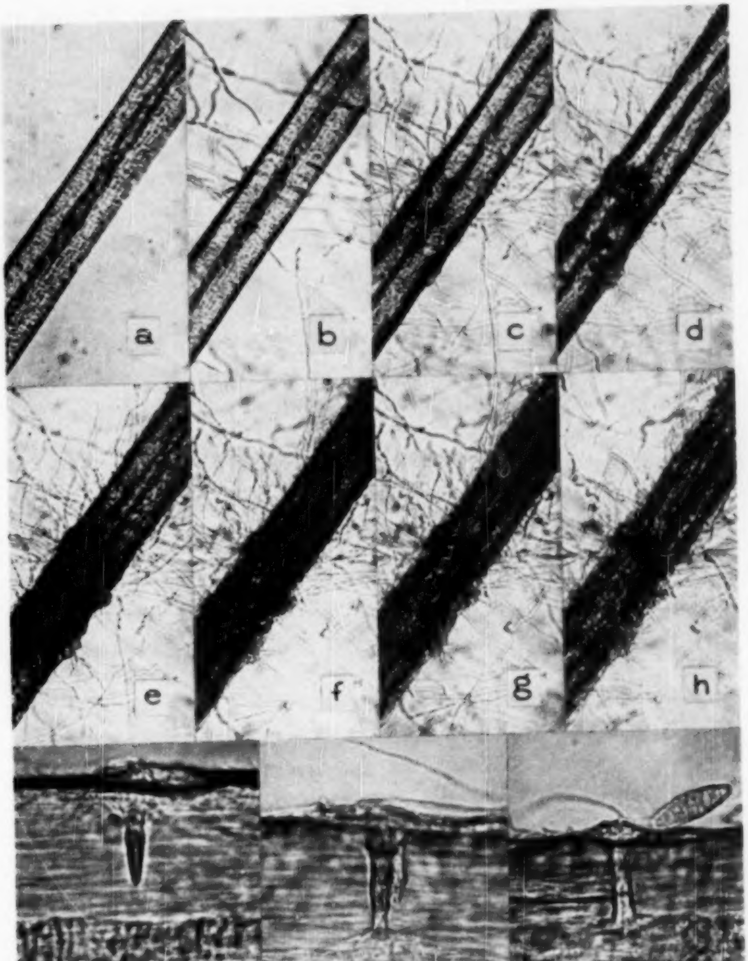

FIG. 2. Human hair attacked by *Microsporum gypseum*.

enclosed the hyphae. The cuticle appeared to be more resistant to the action of the fungus than the cortex, and it was observed to be completely digested in only limited areas.

Human hair. Human hairs are more complex histologically than wool, since they are differentiated into three regions. In addition to the cortex and the cuticle, there is a third region, the medulla, which occupies the central axis of the hair shaft and may be continuous, fragmental, or absent in any one hair. The medulla, when present, is composed of somewhat rounded cells.

A series of photographs showing a hair under attack by *M. gypseum* is shown (FIG. 2, a–h). At the site chosen for study the hair was approximately 115 μ in diameter, and it possessed a continuous medulla. Forty-eight hours after the plate had been inoculated the first hyphae entered the field and gave rise to branches which approached the hair shaft. In some cases the hyphae proceeded along the surface of the hair for some distance. These hyphae were not visible in unstained preparations but could be observed easily in hairs fixed and stained with acid fuchsin in lactophenol. These hyphae which were closely appressed to the hair shaft appeared to be the ones responsible for penetration. The mode of penetration was difficult to establish: in one case a scale of the cuticle appeared to be loosened by mechanical action, but it seems possible that penetration is enzymatic.

Two processes took place almost concurrently after penetration had been effected. First, there was a proliferation of the fungus beneath the cuticle together with an apparent swelling of the cortex, so that a vesicle or eruption was formed. The size of these eruptions varied considerably; sometimes they were relatively small (FIG. 2, i–k), while occasionally they attained a height equal to the diameter of the hair. Second, some of the hyphae digested their way directly into the cortex of the hair to form intrusions which were first conical (FIG. 2, i) and later became cylindrical (FIG. 2, j, k). These intrusions were best studied in hairs fixed and stained with acid fuchsin in lactophenol. The unattacked portions of the hair remained unstained, but the intrusions were deeply stained by the acid fuchsin. In medullated hair the intrusions usually penetrated only as far as the medulla, but occasionally they

appeared to pass through the hair shaft. Breakdown of the cortex appeared to extend longitudinally from the intrusions (FIG. 2, j). The attacked areas of the cortex appeared as dark shadows which gradually extended until the entire hair had lost its original appearance (FIG. 2, h).

Human hair under attack by *M. gypseum* was studied with the polarizing microscope and compared with hair viewed with ordinary light. Normal hair showed strong birefringence when viewed with polarized light. The intrusions, however, showed no birefringence and remained dark when observed through crossed Nichol's prisms in strong contrast to the unattacked portions of the hair. Regions of the hair where the cortex had been attacked showed diminished birefringence at the edges of the zone of attack and no birefringence where the shadows appeared darkest under ordinary light.

DISCUSSION

From a theoretical standpoint, the most significant result of the present investigation was the indication that *Microsporum gypseum* and other dermatophytes are capable of digesting keratin. The almost complete disappearance of particles of horn and fingernails and the loss of birefringence by hair during attack by the fungus indicate that keratin was digested.

Observational evidence for keratin digestion, while not quantitative, is superior in several respects to other possible criteria and is, therefore, a valuable adjunct to them. The criterion of the measurement of the tensile strength of fabric which has been widely used in the study of the degradation of cellulosic textiles is open to objection when applied to multicellular keratinized material, because a decrease in tensile strength might indicate a breakdown of the intercellular cement rather than of the keratinized cells. Similarly, growth of an organism on keratin-containing substrata or the accumulation of by-products of metabolism in a medium might prove misleading unless supported by other evidence.

While keratins are resistant to the action of proteolytic enzymes of the higher vertebrates (*cf.* Routh and Lewis, **1938**), digestion of keratins has been demonstrated with several organisms, notably

the clothes moth (Linderstrøm-Lang and Duspiva, **1935**), the goshawk and vulture (Stanković, Arnovljević and Matavulj, **1929**), and certain Actinomycetes (Jensen, **1930**). Moreover, it seems probable that some of the Chytrids are able to digest keratins, since they appear to be limited in growth to keratinized tissues (Karling, **1948**).

Although contact between hyphae and substratum is not a requisite for digestion, the hyphae must be close to a fragment before it is digested. Even with horn sterilized by autoclaving, the particles showed no sign of digestion until the hyphae had been in the near vicinity of a particle for at least 24 hours. This observation is in agreement with the macroscopic appearance of plates of horn medium, for in no case was the clear zone in the medium observed to extend beyond the margin of the colony. For example, on the horn medium the diameter of 15-day-old colonies of *M. gypseum* averaged 75 mm., while the clear zones averaged only 53 mm. in diameter. This result was in contrast to that obtained on a medium of coagulated casein where the clear zone extended beyond the hyphal tips. These results suggest either that the enzyme responsible for the digestion of keratin does not diffuse rapidly, or that the digestion of the large particles of horn was so slow that the hyphae grew past the fragments before any change in their appearance could be detected. Another possibility, of course, is that the enzyme is oxidized or otherwise inactivated after it has diffused some distance from a hypha, but observations provide no clue as to which of these alternative explanations is valid.

Actual contact of the hyphae with the particle of horn is unnecessary for digestion to occur, because the horn disappeared from within the meshes of the reticulum of branch hyphae which was formed around a fragment of horn. This behavior suggests, therefore, that the enzyme responsible for the digestion of the keratin is not adsorbed on the surface of the hyphae.

With horn there was no evidence of orientation of the hyphae with respect to the orientation of the micelles of the substratum. With wool, the hyphae ran parallel to the orientation of the micelles, but the direction may have been the result of the physical confinement imposed by the cuticle. Hyphae attacking human hair

first ramified over the surface, then penetrated the hair at right angles to the axis, and finally extended into the cortex in a direction parallel to the axis. *Microsporum gypseum,* in its attack on keratin, did not, therefore, show the precise orientation with respect to the direction of the micelles which is characteristic of the attack of certain organisms on cellulose (Stanier, **1942**; Bailey and Vestal, **1937**).

From the point of view of medical mycology, the ability of *M. gypseum* to attack fingernails vigorously *in vitro* is of interest, since this organism does not appear to be an etiologic agent of onychomycosis. Some factor other than the nature of the keratin must be responsible for the distribution of the fungus on the host.

The growth on hair also differs from that observed in the clinic. The spore sheath, which characteristically surrounds the hair shaft in cases of tinea capitis caused by members of the genus *Microsporum,* was absent in the hairs embedded in agar, and both macroconidia (*fuseaux*) and microconidia were produced in abundance on the surface of the medium. Invasion of the hair shaft occurred regularly in these cultures, whereas in cases of tinea capitis caused by *M. gypseum,* the reports are conflicting. Carrión and Silva (**1944**) report that invasion occurs, while Lewis and Hopper (**1943**) state that it does not.

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EXPLANATION OF FIGURES

FIG. 1. Photomicrographs showing representative stages in the digestion of a particle of cows' horn by *Microsporum gypseum*. a, Appearance of particle at time of inoculation. b–f, Appearance of particle after 52, 96, 120, 144, and 196 hours respectively. × 112.

FIG. 2. Photomicrographs showing human hairs attacked by *Microsporum gypseum*. a–h, Successive stages in the attack of *M. gypseum* on a human hair embedded in agar. × 75. a, Hair at time of inoculation. b–h, Photographs taken daily from the second to the eighth day respectively. i–k, Hairs fixed and stained with lactophenol and acid fuchsin. × 425. i, Portion of hair showing eruption of cuticle and intrusion of fungus into cortex. j, Portion of non-medullated hair showing initiation of longitudinal attack. k, Intrusion penetrating to medulla. Note macroconidium of *M. gypseum* adhering to surface of hair.

ASHBYA GOSSYPII—ITS SIGNIFICANCE IN NATURE AND IN THE LABORATORY [1, 3]

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(with 4 figures)

For the past several years investigations concerning the yeast-like fungus *Ashbya gossypii* (Ashby and Nowell) Guilliermond have centered around its capacity to synthesize large amounts of riboflavin, or vitamin B_2. Aside from its flavinogenic potentialities, however, *A. gossypii* possesses certain other characteristics which accentuate its importance to man. Its pathogenicity towards important crop-plants is of considerable economic concern. Its physiological activities are utilized in certain fundamental studies and in a microbiological assay for the vitamin biotin. Finally, its anomalous morphology and cultural behavior present interesting problems relative to its classification and possible phylogenetic relationships. The present report is an attempt to assemble available information concerning these unique characteristics of *A. gossypii* and its potentialities as a vitamin producer.

OCCURRENCE AND PATHOGENICITY

Ashbya gossypii (Ashby and Nowell) Guilliermond, otherwise known as *Nematospora gossypii* Ashby and Nowell, or *Ashbia gossypii* Ciferri and Fragoso (Ashby and Nowell, **1926**; Guilliermond, **1927**, **1928**; Fragoso and Ciferri, **1928**; Ramsbottom, **1942**)

[1] Report of a study made under the Research and Marketing Act of 1946.

[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

[3] A portion of the material here reported was included in a thesis submitted by the senior author to the faculty of the University of Illinois, June 1949, in partial fulfillment of the requirements for the degree of doctor of philosophy in bacteriology.

was possibly first observed by Bartlett in 1907. He noted the characteristic spores of either this organism or *Nematospora coryli* Peglion in infected cotton bolls from British Guiana, but was unable to isolate the organism in pure culture. The first isolation and characterization was recorded by Nowell in 1916–1917 who found the organism to be one of four species infecting cotton in the British West Indies (Nowell, **1915**, **1916**, **1917**, **1917a**). During the same period Nowell (**1917a**, **1918**) obtained further evidence regarding its occurrence. He observed that certain sucking insects were of importance in its dissemination and that tomatoes could also serve as a host. The organism was more fully characterized and given the name *Nematospora gossypii* some years later when it was isolated from the lint and seeds of cotton originating in the British West Indies and Nyasaland, and from the seeds of jimson weed and milkweed (Ashby and Nowell, **1926**).

It has since been reported as a widely distributed and destructive pathogen of a variety of important economic crop-plants, including cotton, coffee, citrus fruits, tomatoes, okra, and various legumes (table 1). It has been isolated in North America only occasionally.

Other possible hosts have been reported by Wingard (**1925**), and the list could undoubtedly be expanded were the host plants of the insects responsible for the transmission of this and related microorganisms thoroughly investigated.

In cotton, the disease produced through infection with *Ashbya gossypii* and related organisms in the genera *Nematospora, Spermophthora,* and *Eremothecium* is variously termed "Stigmatomycosis," "Internal Boll Rot," or "Cotton Staining." The organisms infect the developing lint fibers which become dirty-yellowish in color and mat onto the seeds. The seed coats become stained brown in spots and as the bolls age the locks become totally retarded and the lint is reduced to a papery membrane. The seeds are not penetrated unless mechanical injury occurs. Infection results either in premature dropping of the bolls, or in a drying out of those which remain on the plant. The characteristic symptoms of the disease have been more fully described elsewhere (Pearson, **1934a**, **1947**; Wallace, **1939**; Wickens, **1947**). Tre-

mendous annual losses occur in African cotton-growing regions as a result of this infection.

In coffee, the seeds are affected, the disease appears in the form of

TABLE I

REPORTED HOSTS AND GEOGRAPHIC LOCATION OF *Ashbya gossypii* ISOLATIONS

Reported host	Location
Asclepias curassavica	Trinidad, B.W.I. (1)*
Centrosema plumieri	Belgian Congo (16)
Citrus aurantium	Florida (17)
Citrus nobilis	Florida (17)
Coffea arabica	Belgian Congo (8), Kenya (7, 9), Tanganyika (16)
Coffea robusta	Uganda (16)
Datura metel	Montserrat, B.W.I. (1)
Glycine max	Belgian Congo (16), Union of S. Africa (16)
Glycine soja	Belgian Congo (16), Union of S. Africa (16)
Gossypium spp.	Anglo-Egyptian Sudan (19), Antigua, B.W.L. (1, 11), Bahama Is. (19), Belgian Congo (2, 12, 14, 18, 20), Bequia, B.W.I. (11), Brazil (16), British Guiana (11), Burma (12), Dominican Rep. (3), Grenadine Is., B.W.I. (1), Fiji (19). Jamaica (19), Kenya (19), Montserrat, B.W.I, (1, 11), Natal (19), Nevis, B.W.I. (1, 11), N. Rhodesia (19), Nyasaland (1, 13, 19), Portugese E. Africa (19), Queensland (19), S. Nigeria (6, 19), S. Rhodesia (5, 19), St. Vincent, B.W.I. (1, 11, 19), Swaziland (19), Tanganyika (16, 19), Trinidad, B.W.I. (1), Uganda (4, 19), Union of S. Africa (10, 15, 19)
Hibiscus sp.	Brazil (16)
Hibiscus cannabinus	Uganda (16)
Hibiscus esculentus	Uganda (16)
Lycopersicum esculentum	St. Vincent, B.W.I. (11)
Persea gratissima	Uganda (14)
Phaseolus lunatus	Belgian Congo (16)
Phaseolus mungo	Union of S. Africa (16)
Phaseolus vulgaris	Union of S. Africa (16)
Sida sp.	Brazil (16)
Sterculia platinifolia	Union of S. Africa (16)
Vigna sp.	Belgian Congo (16)

* References refer to citations in table: (1) Ashby and Nowell, 1926; (2) Brixhe, 1938; (3) Fragoso and Ciferri, 1928; (4) Hansford, 1930; (5) Hopkins, 1932; (6) Laycock, 1935; (7) LePelley, 1942; (8) Leroy and Hendrickx, 1941; (9) McDonald, 1932, 1933; (10) Moor, 1930; (11) Nowell, 1915, 1916, 1917, 1917a, 1923, 1939; (12) Rhind, 1927; (13) Richardson, 1941; (14) Steyaert, 1934, 1934a, 1936, 1939, 1946; (15) Ullyett, 1930; (16) Wallace, 1932, 1939; (17) Weber, 1933; (18) Publ. Inst. nat. Etud. agric. Congo belge, 1939, 1941, 1943; (19) Emp. Cott. Grwg. Repts., 1926–1948; (20) Bull. Agric. Congo belge, 1941.

a dry rot with blackening and shrinking of the beans. This leads to "floats" after pulping, and to cracking of the beans in the drying frames (Wallace, **1931, 1932, 1939**). When infections occur in

leguminous plants, the disease is generally termed "Yeast Spot." The seeds become dry, shrivelled, and dark (Wallace, **1932**).

Numerous studies sponsored primarily by the Empire Cotton Growing Corporation of England have demonstrated conclusively that *Ashbya gossypii* and related micro-organisms are transmitted from plant to plant by certain hemipterous insects (see Emp. Cott. Grwg. Corp. Repts., **1926–1948**; Bull. Agric. Congo Belgé, **1941**; Frazer, **1944**; Hansford, **1927**, **1930**; Laycock, **1935**; LePelley, **1942**; Marsh, **1926**; McDonald, **1932**, **1933**; Moor, **1930**; Nowell, **1917a**; Pearson, **1934**; Rhind, **1927**; Steyaert, **1939**, **1946**; Ullyett, **1930**; Wallace, **1932**; Weber, **1933**; Wickens, **1942**; Williams, **1934**). Various species of *Dysdercus* (cotton stainers) have been the most frequently observed carriers, with species of *Nezara* (green bug), *Leptoglossus* (leaf-footed tomato bug), *Phthia* (red tomato bug), *Antestia* (coffee bug), and *Callidea* less often implicated. *Antestia* species have been observed as carriers in all cases of coffee bean infection.

These insects mechanically transmit the organisms from plant to plant during the course of their feeding. The spores or mycelium are carried as external contaminants on the mouth parts and in the deep stylet pouches. Spores apparently do not remain viable or germinate after reaching the intestinal tracts of the insects, but may germinate in the stylet pouches. Spreading of the organisms may be accomplished in two ways. In some cases, spores or mycelia are cast off with the exuviae during moulting and may, by chance, enter an open wound in the plant, the fungus itself being unable to penetrate the outer cells of the host. The most important mode of infection is through injection of the spores by the insects into the tissue of the host. The characteristic needle-shaped spores of *A. gossypii* and related organisms are especially well adapted for this mode of infection. In experimental trials, spraying or surface inoculation of susceptible hosts are usually unsuccessful, while injection by syringe usually results in active infection.

A number of factors affect the degree of infection of cotton bolls, including age and sugar content of the bolls, and humidity. Young bolls are highly susceptible, whereas bolls four to five weeks or older are relatively resistant to infection; the sugar content of bolls

ranges from approximately 50 per cent at one week to less than 1 per cent at maturity, accompanied by a steady decline in susceptibility; high humidity increases the incidence of infection (Marsh, **1926**; Emp. Cotto. Grwg. Repts., **1926–1948**; Hopkins, **1932**; Pearson, **1934, 1939, 1947**; Steyaert, **1936, 1938, 1939**; Rainey, **1940, 1948**; Wickens, **1947**).

The staining of cotton by these organisms is due apparently to a modification of the protoplasm of the central canal of the lint hairs (Marsh, **1925**). Pearson (**1934**) enlarged upon this theory by indicating that such a modification followed killing of the fibers by a toxin secreted by the organisms. He reported that decrease in susceptibility with age of the bolls was due to evacuolation of the lint hairs and drying which impeded the movement of toxic substances within the hairs. In later experiments Pearson (**1947**) was unable to redemonstrate toxin production by *A. gossypii.*

The fact that *A. gossypii* is an important plant pathogen has caused some concern regarding its use in an industrial process for the production of riboflavin. However, our attempts to isolate viable cultures from drum- or spray-dried fermentation residues have been unsuccessful. In addition, preliminary thermal-death-time studies have indicated that the organism is rather susceptible to moderate temperatures under laboratory conditions, being killed in less than 20 minutes at 60° C and in less than 5 minutes at 70° C when exposed in fermented liquors.

TAXONOMY AND RELATIONSHIPS

Ashbya gossypii offers a challenge to the taxonomist, since it appears to possess characteristics of both phycomycetous and ascomycetous fungi. Its anomalous morphology presents problems in classification and poses questions as to its phylogenetic relationships.

Nowell (**1917**) first pointed out the close relationship of this organism to certain species of *Nematospora, Eremothecium,* and what is now the genus *Spermophthora.* He indicated that not until spore formation occurred could these three genera be separated. *Ashbya gossypii* most closely resembled *Nematospora* in the mode of formation and morphology of its spores, but differed

in its strict adherence to a mycelial habit whereas species of *Nematospora* grew primarily as yeast-like forms. He regarded the sporiferous sacs of these organisms as sporangia and for that reason felt that the genus *Nematospora* should not be included among the Saccharomycetes. At this time he designated *A. gossypii* simply as species "C." Wingard (**1925**) further noted the relationships between species C and various *Nematospora* species but, following cytological studies of *N. phaseoli,* he advanced the opinion that the sporiferous sacs were, in fact, asci, and that these organisms were Saccharomycetes. He pointed out that species C appeared to have characteristics in common with such genera as *Nematospora, Monospora, Coccidiascus, Eremothecium, Protascus,* and certain species of *Endomyces.* Ashby and Nowell (**1926**) later characterized and named species C *Nematospora gossypii,* based on the close conformity of its sporiferous sacs to those of other species of *Nematospora,* and on the specialized spores. However, the usual mycelial habit of *A. gossypii* was in direct contradiction to the yeast-like habit of *Nematospora* species generally. They doubted that the organism was a Saccharomycete and left open the question of the exact nature of its sporiferous sacs. They pointed out the common salient features of the three genera, *Spermophthora, Nematospora,* and *Eremothecium.*

In a series of papers devoted to the cytology and phylogeny of the lower Ascomycetes, Guilliermond (**1927**, **1928**, **1935**, **1936**) suggested placing the organism in the *Hemiascomycetes* but assigned to it a new genus name, *Ashbya.* This suggestion was based on his observations of spore formation at the expense of a sporoplasm, the presence of an epiplasm, even numbers of spores, and two mitoses of the nuclei prior to spore formation. He considered the fruiting body of *A. gossypii* as intermediate between a sporangium and an ascus because of its multinucleate nature, and pointed out that the organism was related to such genera as *Dipodascus, Protomyces, Endogone, Taphridium, Spermophthora,* and *Eremothecium.*

Fragoso and Ciferri (**1928**) increased the synonomy still further by presenting a Latin description of the organism under the generic name *Ashbia.* They considered the organism to be a Saccharomycete.

Stelling-Dekker (**1931**) omitted the genus *Ashbya* from her classification of the sporogenous yeasts, and considered its position doubtful, representing no more than a section of the genus *Nematospora* in the subfamily Nematosporoideae and family Endomycetaceae. According to her treatment, it would be related to the genera *Monosporella* and *Coccidiascus.*

Dodge (**1935**) retained the generic name *Ashbya,* but set up a separate family, Ashbyaceae, under the order Endomycetales to include this genus, as well as the genera *Piedraia* and *Eremothecium.*

It would thus appear that taxonomists generally agree upon placing the organism in the order Endomycetales of the Hemiascomycetes. From this point on, further attempts to classify *Ashbya gossypii* differ. It appears to possess characteristics of several families. If one accepts Martin's system of classification (**1948**), *A. gossypii* is seen to possess characteristics which suggest relationships with three different families. In the following quoted characterizations, features which apply to *A. gossypii* are italicized:

"Ascoideaceae—*Spore sacs (asci?) many spored;* gametangia, when present, sometimes multinucleate." Such genera as *Ascoidea* and *Dipodascus* are included here.

"Endomycetaceae—Asci with 8 ascospores or fewer; gametangia when present always uninucleate; *asci borne on a well developed mycelium.*" This includes the genera *Endomyces* and *Eremascus.*

"Saccharomycetaceae—Mycelium lacking, reproduction by budding, *asci formed by transformation of a single cell,* or as the result of fusion of two cells." Such genera as *Saccharomyces, Pichia,* and *Nematospora* are included in this family.

Recently, Routien (**1949**) has described a new genus, *Crebothecium,* designed to include the organism now known as *Eremothecium ashbyii.* *Ashbya gossypii* closely resembles *E. ashbyii* and with the exception of the morphology and arrangement of the spores within the sporiferous sacs, the two species could well be included in the same genus. The possibility that *A. gossypii* actually represents a species within the genus *Eremothecium,* or the new genus *Crebothecium,* if such genus is valid, is not without consideration. There is the further possibility that *E. ashbyii* could represent a species within the genus *Ashbya* since the latter has been validly described. Guilliermond (**1936**) has pointed out

the essential differences between *E. ashbyii* and *A. gossypii.* The former organism has broader, shorter spores which are curved at the needle-like extremity, beveled at the opposite end, and lack elongate appendages. They are not arranged in any predetermined order, *i.e.*, there are no fascicles of spores. In addition, the central portion of the spores of *E. ashbyii* give a positive test for pectin, while those of *A. gossypii* do not. Septa are more numerous in *A. gossypii* than in *E. ashbyii,* whereas the dichotomy of the mycelium of the latter is more regular. *E. ashbyii* appears to be more fastidious in its nutritional requirements. According to the philosophy of the investigator, these differences could be regarded or disregarded in establishing nomenclature.

Further study of the characteristics and relationships of these and related genera seems warranted not only from an academic but also from a practical standpoint, since certain of the species within this group are of considerable economic importance, patents covering the production of riboflavin being centered around particular species.

CULTURAL AND MORPHOLOGICAL CHARACTERISTICS

The cultural characteristics of *Ashbya gossypii* have been described by a number of investigators, including Nowell (**1917**), Ashby and Nowell (**1926**), Guilliermond (**1927, 1936**), Stelling-Dekker (**1931**), Wallace (**1932**), Frazer (**1944**), and Wickerham *et al.* (**1946**). The following characterization is based upon these reports. *A. gossypii* grows freely on potato, potato-glucose agar, potato-sucrose agar, beer-wort, gelatin, and carrot, producing at first a spreading, moist, appressed hyphal growth with short, pointed, matted hair-like outgrowths over the surface. After a week on potato, scattered pustular dots appear which unite to form a folded, vermiform, firmly gelatinous plechtenchyma. The pigmentation of the substrate remains unchanged. On potato-glucose or potato-sucrose agar a similar hyphal growth appears which changes to a moist, translucent, dirty-white, folded and wrinkled plechtenchyma which can be readily peeled off.

Based upon our studies of a flavinogenic strain (FIG. 1A) of the organism, the following colony description may be taken as typical of riboflavin producing strains:

Colonies upon yeast extract-malt extract-peptone-glucose agar after 10 days at room temperature (24–26° C), circular, flat to slightly raised, membranous, myceloid with advancing edge filamentous. On the second or third day of growth short, pointed, matted, hair-like growths form on the surface of the colony. On the fourth or fifth day, radial ridges or grooves appear, and in some instances haphazard wrinkles develop. Colonies grow at a fairly uniform rate of 3 to 5 mm. per day, reaching a maximum diameter of 38 to 50 mm. in 10 days, usually 35 to 40 mm. The marginal edges of colonies are made up of typical dichotomously branched hyphae (FIG. 2B). Colonies, at first, completely white

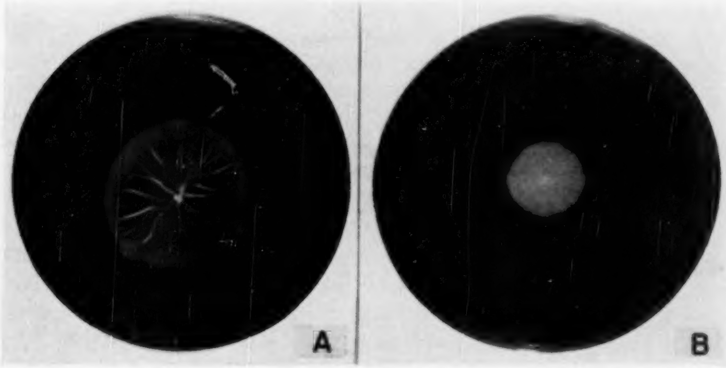

FIG. 1. *Ashbya gossypii* (Ashby and Nowell) Guilliermond. Ten-day colonies growing on peptone, yeast extract, malt extract, glucose medium; room temperature. *A*, Strain NRRL Y-1056 (flavinogenic). *B*, Strain NRRL Y-985 (non-flavinogenic).

to greyish-white, becoming pale lemon yellow in 2 to 3 days, most intense at the center. At 5 days, the pigment is intensified to a lemon yellow and at 10 days to an orange yellow to reddish orange. The advancing edges of the colonies at all times are white for approximately 5 mm. Depending upon the rapidity of riboflavin synthesis on solid media, concentric rings begin to appear marking zones of periodically increased flavinogenesis. Sectoring to white areas is infrequently noted. In 7 to 10 days, the medium surrounding the colony is often pigmented a yellowish-green due to the diffusion of riboflavin or some derivative. Colonies are extremely adherent and may be peeled from the surface of the medium in their entirety.

In our experience non-flavinogenic strains of *Ashbya gossypii* duplicate the above except for the absence of pigmentation and a reduced tendency toward the formation of radial ridges, grooves, or wrinkling (FIG. 1B).

In wort and other liquid media, mycelial flocs are generally formed in a clear liquid. A slimy precipitate has been observed

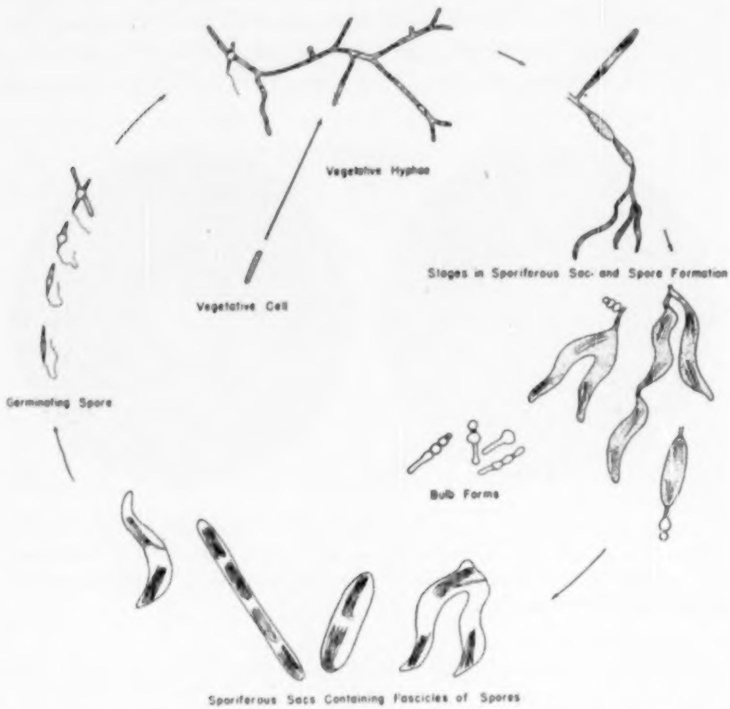

FIG. 2. Schematic presentation of the life-cycle of *Ashbya gossypii* (Ashby and Nowell) Guilliermond.

in some cases. Under agitation and aeration in liquid media, the culture becomes very homogeneous and tends to rather rapid autolysis after 7 days. Flavinogenic strains are characterized by the production of lemon yellow to orange pigment in certain media most intense when cultures are under aeration.

Descriptions of the morphology of *Ashbya gossypii* have been recorded by Nowell (**1917**), Ashby and Nowell (**1926**), Guillier-

mond (**1927**, **1928**, **1936**), Stelling-Dekker (**1931**), and Wallace (**1932**). From these studies and our own observations, the following morphological characteristics may be considered as typical of this organism (FIGS. 2, 3, 4):

Hyphae hyaline, often vacuolated or containing a granular material and numerous hyaline droplets, at first non-septate. The first septum develops 150 to 200 μ behind the growing tip, others forming at intervals of 50 to 70 μ. Hyphae converging slightly at the septa, developing into long thin segments from 6 to 10 μ in diameter, branching dichotomously. Lateral buds developing on the hyphae at an early stage of growth, singly or in short chains, falling away and reported to be capable of germination. Vegetative reproduction reported to occur through transverse fission and subsequent separation of the daughter cells, this toruloid form being assumed only under conditions which preclude spore formation. In flavinogenic strains, riboflavin or a derivative may be observed within certain cells as a yellowish-oily fluid, or as needle-like orange crystals (FIG. 3D). The number of nuclei in the cells varies.

Sporiferous sacs (asci?, sporangia?) abundant, developing from hyphal segments, appearing haphazardly along the mycelium, occurring singly, or in chains, sometimes branched. These cells vary considerably in size, but range predominantly from 100 to 200 by 10 to 20 μ. They occur in a variety of shapes, sometimes clavate or cylindrical, but most frequently in the form of a sigmoid body. Glycogen is abundant at the beginning of spore formation and the sporiferous sacs may contain a granular protoplasm or may be highly vacuolated. Later, the interior of the sacs becomes hyaline except for the space occupied by the spores. The sacs ultimately break or autolyze to release fascicles of mature spores.

The nuclei in the sacs are reported to undergo two mitoses in spore formation similar to those of the higher Ascomycetes. Guilliermond (**1927**, **1928**) has described in detail the cytological processes involved. At the end of the nuclear divisions, a sporogenous plasma remains. This material then divides into long filaments, reuniting into fascicles as the spores are formed.

Mature spores are grouped parallel into two or more fascicles of 2 to 6 spores, and are arranged lengthwise in the sac. The spores, when liberated, are held together by means of long intertwined appendages 50 to 100 μ in length, consisting of a difficultly stainable homogeneous plasma. Spores may number from 4 to 32 per sac (usually 12 to 16). Dependent upon the substrate in or upon which the organism has been cultivated, spores vary in dimensions from 25 to 37 by 2 to 5 μ. The spores are acicular to fusi-

form. They are described by Guilliermond as having an anterior region occupied by structures resulting from a degeneration of the cytoplasm, a central region made up of very dense cytoplasm containing the nucleus and giving a negative test for pectin, and a

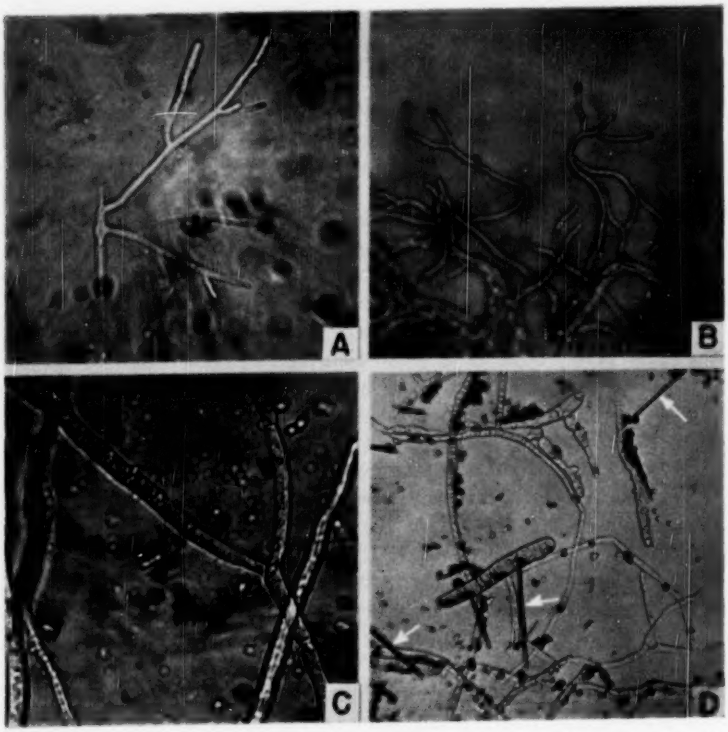

FIG. 3. *Ashbya gossypii* (Ashby and Nowell) Guilliermond (Strain NRRL Y-1056). *A*, *B*, and *C*. Preparations from 10-day colony, room temperature. *A*, Germinating spore, × 750. *B*, Typical dichotomous branching of hyphae, edge of colony, × 200. *C*, Typical hyphae showing septa, dichotomous branching, and granular nature of contents, × 1500. *D*, Typical preparation from fermented substrata showing crystals of riboflavin outside cells (arrows) and "bulb-forms" (upper center), × 750.

polar region consisting of the filiform appendage. In the center of the spore a thin septum may be observed occasionally. In germinating, a globular expansion occurs in the central nucleate region and from this arise one or more germ-tubes which ultimately develop into hyphae (FIGS. 2, 3).

Swollen hyphal elements, termed "Bulb-forms" (FIGS. 2, 3, 4), are typical of the flavinogenic strains of *Ashbya gossypii*. These occur in cultures where riboflavin has been synthesized. They

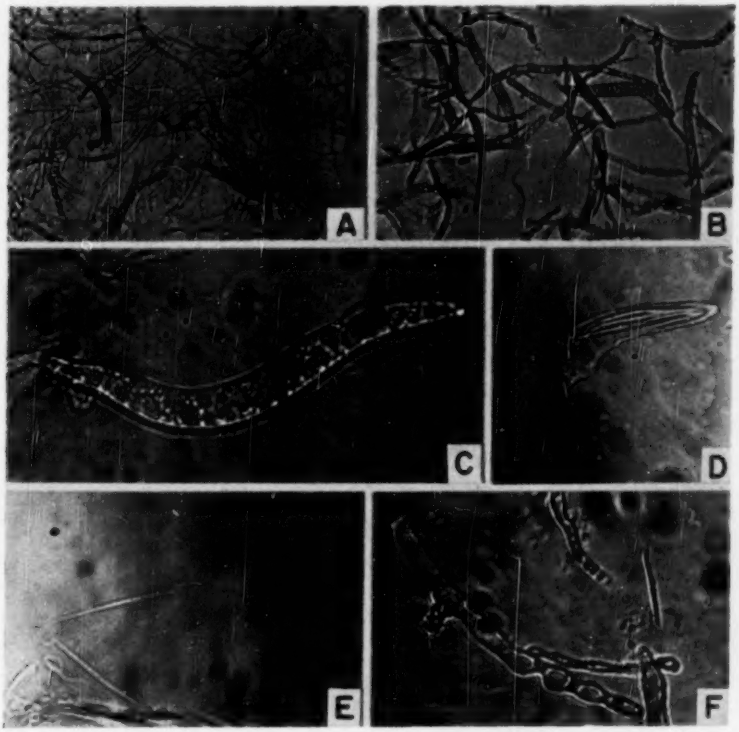

FIG. 4. *Ashbya gossypii* (Ashby and Nowell) Guilliermond (Strain NRRL Y-1056). Preparations from 10-day colony, room temperature. *A* and *B*, typical mounts showing nature of hyphae, immature sporiferous sacs, "bulb-forms" and sporiferous sacs containing spores, × 300. *C*, Sporiferous sacs at end of chain, showing characteristic sigmoid form and granular nature just prior to spore formation, × 1500. *D*, Sporiferous sac containing one fascicle of spores, second fascicle has been released through pore at lower end of sac, × 1300. *E*, Single spore, showing septum and portion of appendage, × 1300. *F*, "Bulb-forms," × 1300.

possibly represent the response of the organism to changes in osmotic tension influenced by the production of large amounts of the vitamin. These cells are usually filled with a yellowish-oily fluid, probably riboflavin or some derivative in the dissolved state.

They vary considerably in size and appearance and develop either as terminal or intercalary cells. In some cases there is a distinct resemblance to chains of yeast-like cells.

PHYSIOLOGICAL CHARACTERISTICS

The nitrogen requirements of *Ashbya gossypii* have been investigated rather extensively. Farries and Bell (**1930**) found that KNO_3 and NH_4^+ salts were of little value as nitrogen sources. Their findings were confirmed by Buston *et al.* (**1938**) who observed no utilization of the ammonium salts of sulfuric, nitric, hydrochloric, tartaric, lactic, or pyruvic acids in mineral-salts media containing inositol and a factor obtained from lentils by Buston and Kasinathan (**1933**). When these media were supplemented with β-alanine or *l*-aspartic acid, moderate growth was attained. Stelling-Dekker (**1931**) and Wickerham (personal communication) have observed that NO_3^- is not assimilated by either flavinogenic or non-flavinogenic strains.

Farries and Bell (**1930**) further demonstrated that the organism is capable of growing in media containing the hydrolytic products of natural proteinaceous materials such as degraded animal protein, egg-white, gluten, or casein. Their strains failed to grow in media containing gelatin, edestin, or fibrin in any form, or in media containing simple mixtures of amino acids. Growth was obtained with certain of these latter materials when peptone, or a factor easily separable by alcoholic precipitation from egg-white, or casein was present. Buston and Pramanik (**1931**) and Buston *et al.* (**1938**) in confirming these findings identified one of the accessory factors as *meso*-inositol. With media containing *meso*-inositol and the lentil factor of Buston and Kasinathan (**1933**), *Ashbya gossypii* was shown capable of utilizing simple mixtures of amino acids, asparagine, or ammonium aspartate as nitrogen sources. Tryptophane was found to be non-essential. Stelling-Dekker (**1931**) has reported that the organism can feebly attack gelatin, as shown by liquefaction after 42 days' incubation.

It was ultimately determined in a series of studies by Buston and Pramanik (**1931**), Buston and Kasinathan (**1933**), Kögl and Fries (**1937**), Fries (**1938**), and Schopfer (**1944**) that in addition

to *meso*-inositol, biotin and thiamin were required for adequate growth of *A. gossypii*. Of these factors, biotin (the lentil factor of Buston and Kasinathan) was found to be the most important. Thiamin is synthesized by the organism, but in sub-optimal amounts. Good growth was obtained in glucose-mineral salts media in the presence of the three factors. Attempts to replace the biotin with pimelic acid or with a mixture of pimelic acid and *l*-cystine have been unsuccessful (Robbins and Ma, **1942**).

The biotin requirement is such that a microbiological assay for this factor has been developed, based on the fact that within certain ranges, the weights of mycelial pads are directly proportional to the concentration of biotin in the medium (Robbins and Schmidt, **1939**; Robbins, **1940**). Their basal medium consists of dextrose, asparagine, mineral-salts, *meso*-inositol, thiamin, and trace elements. For biotin assay, cultures were incubated at 23 to 25° C for 9 days, the mats weighed, and the biotin content of samples calculated from a standard curve.

Ashbya gossypii has also proved useful in other physiological studies, *e.g.*, the mechanism of the physiological activity of gammexane (hexachlorocyclohexane). The γ-compound was shown to markedly decrease growth of the organism while the α- and β-isomers had little effect. This confirmed the hypothesis that the activity of the γ-isomer is due to its antagonism toward the essential metabolite *meso*-inositol, considered to have the same spatial configuration (Buston *et al.*, **1946**). Hawker (**1948**) has employed *A. gossypii* in mixed culture with *Melanospora destruens* to prove the latter capable of synthesizing inositol. Buston and Roy (**1949**) have used the organism in a study of the mechanism of action of certain unsaturated lactones. Their evidence indicates that the inhibition of growth caused by these compounds is not concerned with the metabolism of thiamin, ascorbic acid, nicotinic acid, biotin, or inositol. They further demonstrated that certain sulfhydryl compounds and amino acids may counteract the effects of these lactones.

The carbon requirements of *Ashbya gossypii* have been studied by several investigators. Marsh (**1926**) observed that the organism was unable to attack potato starch in a mineral-salts medium.

Ashby and Nowell (**1926**) reported it as having little diastatic ability and Farries and Bell (**1930**) and Wickerham (personal communication) have indicated that the organism may attack soluble starch only weakly. Glucose, fructose, and sucrose have been shown to serve as adequate carbon sources. Maltose and cellobiose are assimilated less readily. Rhamnose, arabinose, xylose, galactose, lactose mannitol, and inulin, however, do not serve as adequate carbon sources in peptone or amino acid-mineral salts-vitamin media (Farries and Bell, **1930**; Wickerham, personal communication).

Stelling-Dekker (**1931**) has reported that *A. gossypii* is unable to ferment glucose, fructose, mannose, galactose, sucrose, maltose, or lactose, when measured in terms of gas production. Ethyl alcohol was shown to support slight growth when employed as a substrate.

Marsh (**1926**) and Pearson (**1947**) have reported that the organism is unable to attack cellulose.

Farries and Bell (**1930**) observed traces of ethyl alcohol in certain media in which *A. gossypii* has been cultivated.

Mickelson (**1948**) has stated that the end products of glucose metabolism of *A. gossypii* are cells and CO_2. He further indicated that pyruvate, acetate and ethyl alcohol are readily oxidized, whereas lactate and succinate are only slowly oxidized.

RIBOFLAVIN PRODUCTION

As a physiological function of potentially great economic importance, certain strains of *Ashbya gossypii* possess the ability to synthesize large quantities of the vitamin riboflavin. As early as 1930 Farries and Bell noted the production of yellow pigment by certain strains, whereas it was not identified as riboflavin until 1935 (Guilliermond *et al.*). Until 1946, only traces of pigment were observed, principally in old cultures. It remained for Wickerham *et al.* (**1946**) to demonstrate substantial riboflavin synthesis in a strain earlier received from Dr. W. J. Robbins, New York Botanical Gardens. Following this observation and report, *Ashbya gossypii* NRRL Y-1056 has been developed by Tanner and coworkers (**1947**, **1948**, **1949**) and by the authors (**1949**) to a point

where yields up to 1760 μg. of riboflavin per ml. have been obtained in shake-flask cultures in a peptone-corn steep liquor-glucose medium. These maximum yields have been attained through feeding additional glucose during the course of the fermentation (Pridham, **1949**). Yields of 500 to 600 micrograms per ml. are regularly achieved in pilot-plant trials by submerged aerobic fermentation using animal stick liquor, corn steep liquor, and commercial glucose (Tanner *et al.*, **1947**, **1948**, **1949**; Langford, **1948**; Pfeifer *et al.*, **1949**). Fermented media are ultimately dried down to give riboflavin concentrates containing from 25,000 to 30,000 μg. of the vitamin per gram. This material is employed directly in supplementing animal feeds. By appropriate chemical or microbiological techniques the riboflavin may also be recovered to meet pharmaceutical standards.

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PATHOGENIC SPOROTRICHA; THEIR CARBOHYDRATE REACTIONS

H. I. LURIE *

In 1809, Link (**10**) described eleven species of *Sporotrichum*, most of which were derived from decaying wood.

It was not until 1898 that Schenck (**16**) described a fungus resembling a *Sporotrichum* isolated from a human lesion. One year later Brayton (**4**) reported a case which clinically accorded with sporotrichosis but cultural examination was not done. In 1900, Hektoen and Perkins (**9**) isolated from a patient a fungus which was found to be identical with that of Schenck. They named the organism *Sporotrichum Schenckii*.

Three years later de Beurmann and Ramond (**5**) described the first case of sporotrichosis in France. This strain was studied by Matruchot and Ramond (**11**). They named it *Sporotrichum Beurmanni*. During the next few years numerous cases were reported in the world literature.

Several pathogenic species of *Sporotrichum* have been reported. Some descriptions include the carbohydrate fermentations, *e.g.*, *S. Grigsbyi* is said to be inert in glucose, sucrose and lactose, while *S. Fonsecai* is said to produce acid with glucose, fructose and maltose. Schenck (**16**) as well as Hektoen and Perkins (**9**) report that *S. Schenckii* produces no gas in lactose, glucose or saccharose broth. Acid production is not mentioned. Gougerot and Blanchetière (**8**) state that all *S. Beurmanni* strains studied by them ferment saccharose but not lactose and that the original *S. Schenckii* strain hydrolyses and ferments lactose but not saccharose.

In 1915 Meyer and Aird (**12**) carried out an extensive investigation of the carbohydrate fermentations of fifteen strains of *Sporotrichum*. When grown in Erlenmeyer flasks for 60–90 days in the presence of a litmus indicator all strains were found to produce acid but no gas in glucose. No strains fermented lactose or man-

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nite. Other sugars gave variable results. When twelve of the strains were grown for several months, the acid produced was estimated by titrating with N/20 NaOH. All were found to ferment glucose with the production of lactic acid. Lactose was not fermented by any strain in 68 days, but after 270 days all strains produced a trace of acid. *S. Beurmanni* did not ferment saccharose. They conclude by saying that the differentiation of species by carbohydrate fermentations is impossible.

In many reports the technique adopted to determine whether a sugar was fermented or not is not described. Most workers depend on the production of gas and the change of pH to acid as shown by indicators. In a few of the more intensive studies the total amount of free acid was estimated by titration with dilute alkali and in one or two cases the acid was identified.

Since ammonia and potassium bicarbonate are catabolic products of fungus growth both the pH of the medium as shown by indicators and the total amount of free acid as shown by titration will depend on the balance between the amount of acid produced in the fermentation of a carbohydrate and the amount of catabolic products excreted by the growing fungus. It is possibly due to these factors that conflicting reports appear in the literature.

With this in mind, it was thought that a more detailed approach was necessary.

Seven strains of *Sporotrichum* were selected for this study:

No. 8. *S. asteroides.* Splendore. Westerdijk, Baarn. June 1941.
No. 13. *S. Schenckii.* Duke University. February 1944.
No. 20. *Rhinocladium Beurmanni.* Dr. Langeron, Paris. 1934.
No. 21. *Rhinocladium equinum.* Sabouraud's collection. 1934.
No. 32. *S. Beurmanni.* South Africa. Isolated from patient who contracted disease in gold mine. 1947.
No. 61. *S. Beurmanni.* South Africa. Isolated from timber in same gold mine. 1947.
No. 72. *S. tropicale.* Dr. Ghose, India.

The first experiment was to note changes in pH of the medium as shown by color changes of indicators. Various media and indicators were tried. A medium containing Casamino-acids with

TABLE 1

ACID PRODUCTION OF SPOROTRICHUM SP. WITH VARIOUS CARBOHYDRATES ON A SOLID HYDROLYSED CASEIN MEDIUM. ORIGINAL pH = 6.8. BROMO-CRESOL-PURPLE INDICATOR. 37°C

Strain	Glycerine	Glucose	Mannite	Saccharose	Maltose	Lactose	Starch	Glycerine	Glucose	Mannite	Saccharose	Maltose	Lactose	Starch
	4 days							5 days						
8	++	±	+	−	−	−	−	++	±	+	−	−	−	−
13	±	+	+	−	−	−	−	+	++	+	−	−	−	−
20	+	±	+	−	−	−	−	++	±	++	−	−	−	−
21	++	++	++	−	−	−	−	++	++	++	−	−	−	−
32	++	++	++	−	−	−	−	++	++	++	−	−	−	−
61	+	+	−	−	−	−	−	++	++	−	−	−	−	−
72														
	7 days							9 days						
8	++	−	±	−	−	−	−	++	−	−	−	−	−	−
13	+	+	±	−	−	−	−	++	+	−	−	−	−	−
20	++	−	+	−	−	−	−	++	−	±	−	−	−	−
21	++	++	++	−	−	−	−	++	++	++	−	−	−	−
32	++	++	++	−	−	−	−	++	++	++	−	−	−	−
61	++	+	−	−	−	−	−	++	+	−	−	−	−	−
72														
	10 days							15 days						
8	++	−	−	−	−	−	−	++	−	−	−	−	−	−
13	++	−	−	−	−	−	−	++	−	−	−	−	−	−
20	++	−	−	−	−	−	−	++	−	−	−	−	−	−
21	++	++	++	−	−	−	−	++	−	−	−	−	−	−
32	++	++	++	−	−	−	−	++	++	++	−	−	−	−
61	++	−	−	−	−	−	−	++	−	−	−	−	−	−
72														
	21 days							24 days						
8	++	−	−	−	−	−	−	++	−	−	−	−	−	−
13	++	−	−	++	++	−	−	++	−	−	++	++	−	−
20	++	−	−	−	−	−	−	++	−	−	−	−	−	−
21	++	−	−	−	−	−	−	++	−	−	−	+	−	−
32	++	++	++	−	−	−	−	++	++	++	−	−	−	−
61	++	−	−	++	−	−	−	++	−	−	++	−	−	−
72	−	−	±	−	−	−	−	−	−	+	−	−	−	−
	28 days							31 days						
8	++	−	−	−	−	−	−	++	−	−	−	−	−	−
13	++	−	−	++	++	−	−	++	−	−	+	±	−	−
20	++	−	−	−	−	−	−	++	−	−	−	−	−	−
21	++	−	−	−	++	−	−	++	−	−	−	++	−	−
32	++	++	++	−	−	−	−	++	++	++	−	−	−	−
61	++	−	−	−	−	−	−	++	−	−	−	−	−	−
72	−	−	±	−	−	−	−	−	−	+	−	−	−	−

++ = strongly acid. + = acid. ± = trace of acid. − = no acid.

l-cystine, nicotinic acid and agar was found to give the most satisfactory growth. Bromo-cresol-purple was used as an indicator. At a pH of 6.8 it is a definite purple color whereas below pH 6 it is bright yellow. Into the basic medium were incorporated various carbohydrates in a concentration of 1 per cent. The pH in each case was adjusted to 6.8. Slopes were inoculated with the different strains of *Sporotrichum* and maintained at 37° C. All cultures were examined daily and the color changes recorded. The results are summarized in table 1.

Strain No. 72 showed very poor growth. It will be seen that of the other six strains, all produced acid in glycerine and glucose, five in mannite, two each in saccharose and maltose and no strain produced acid in lactose or starch.

It is worthy of note that whereas on the fifth day all strains (except No. 61 in mannite) showed the presence of acid in glycerine, glucose and mannite, by the fifteenth day glycerine was still acid with all strains, whereas glucose and mannite had reverted to their original pH with all strains except No. 32. It will also be seen that acid appeared for the first time in maltose and saccharose (strains 13 and 61) only after twenty-one days.

As the changes in pH appeared to vary with the length of time, it was thought advisable to see if these results were reproducible. Consequently the experiment was repeated and a few more carbohydrates were included. For the second experiment strain No. 72 was kept at room temperature and a satisfactory growth was obtained. The results are summarized in table 2. All seven strains produced acid in glycerine, glucose, mannite and levulose, three strains produced acid in sorbite, dextrine, adonite and inulin, two produced a trace of acid in arabinose and dulcite.

By comparing tables 1 and 2 it will be seen that the results are not entirely comparable and that there is nothing constant about the time taken for the acid to appear or disappear, *e.g.*, in the first experiment strain No. 61 did not produce acid in mannite but it did do so in the second experiment. In the first experiment strain No. 20 produced acid in glycerine on the fourth day but in the repeat experiment it did not appear until the twelfth day. In the first experiment strain No. 21 showed much acidity in glucose from the fourth to the tenth day and by the fifteenth day the

acidity had disappeared. In the second experiment acid was demonstrable on the fourth day only and had disappeared by the fifth day.

TABLE 2

ACID PRODUCTION OF SPOROTRICHUM SPP. WITH VARIOUS CARBOHYDRATES ON A SOLID HYDROLYSED CASEIN MEDIUM. ORIGINAL pH = 6.8. BROMO-CRESOL-PURPLE INDICATOR. STRAINS 8 TO 61 AT 37°C. STRAIN 72 AT ROOM TEMPERATURE

Strain	3 days										5 days									
	Glycerine	Glucose	Mannite	Laevulose	Sorbite	Adonite	Dextrine	Inulin	Arabinose	Dulcite	Glycerine	Glucose	Mannite	Laevulose	Sorbite	Adonite	Dextrine	Inulin	Arabinose	Dulcite
8	++	−	−	+	−	−	−	−	−	−	++	−	−	−	−	−	−	−	−	−
13	++	++	−	++	−	−	−	−	−	−	++	++	−	++	−	−	−	−	−	−
20	−	−	−	−	−	−	−	−	−	−	−	−	−	±	−	−	−	−	±	−
21	++	+	−	++	−	−	−	−	−	−	++	−	+	++	−	−	−	−	−	−
32	++	++	++	−	++	−	−	−	−	−	++	++	++	++	++	+	−	−	−	−
61	++	++	−	++	−	−	−	−	+	−	++	++	++	++	−	−	−	−	±	−
72	−	−	−	+	−	−	−	−	−	−	++	++	−	++	−	−	−	−	−	−
	8 days										12 days									
8	++	−	−	−	−	−	−	−	−	−	++	−	±	−	−	−	−	−	−	−
13	++	++	±	++	−	−	+	−	−	−	++	++	−	++	−	−	++	−	−	−
20	−	−	−	−	−	−	−	−	−	−	++	++	±	−	−	−	−	−	−	−
21	++	−	++	++	++	−	−	−	−	−	++	−	++	++	++	−	−	−	−	−
32	++	+	++	++	++	++	−	−	−	−	++	−	++	++	++	++	−	−	−	−
61	++	++	++	++	−	−	−	−	+	−	++	++	−	++	−	−	−	++	−	−
72	++	++	++	++	++	−	++	−	−	−	++	++	++	++	++	+	++	−	−	−
	17 days										21 days									
8	++	−	±	−	−	−	−	−	−	−	++	+	++	−	−	−	−	−	−	−
13	++	++	−	++	−	−	++	±	−	−	++	++	−	++	−	−	++	++	−	−
20	++	++	±	−	−	−	−	−	−	−	++	++	±	−	−	−	−	−	−	−
21	++	−	++	++	++	−	++	−	−	−	++	−	++	++	++	−	++	−	−	−
32	++	−	++	++	++	++	−	−	−	−	++	−	++	++	++	++	−	−	−	−
61	++	++	−	++	−	−	−	++	−	−	++	++	−	++	−	−	−	++	−	−
72	++	++	++	++	++	++	++	−	−	−	++	++	++	+	++	++	++	−	−	−
	25 days										31 days									
8	++	++	++	−	−	−	−	−	−	−	++	++	++	−	−	−	−	−	−	−
13	++	++	−	++	−	−	++	++	−	−	++	++	−	++	−	+	++	++	−	±
20	++	++	+	−	−	−	−	±	−	−	++	++	++	−	−	−	−	−	−	±
21	++	−	++	++	++	−	++	−	−	−	++	−	++	++	++	−	++	−	−	−
32	++	−	++	++	++	++	−	−	−	−	++	−	++	++	++	++	−	−	−	−
61	++	++	−	++	−	−	−	++	−	−	++	++	−	++	−	−	−	++	−	−
72	++	++	++	−	++	++	++	−	−	−	++	++	++	−	++	±	++	−	−	−

++ = strongly acid. + = acid. ± = trace of acid. − = no acid.

It is possible, therefore, that some of the discrepancies in the literature are due to differences in the time the fungi are allowed to grow before the pH value is noted. These times vary with different authors from a few days to nine months. Obviously, therefore, the production of acid as shown by the color change of an indicator cannot be accepted as evidence of a carbohydrate

being fermented unless numerous other factors are carefully standardized.

The titration of the amount of acid produced is subject to the same fallacies. The only certain method of determining whether a carbohydrate has been fermented or not is the actual estimation of the amount of carbohydrate present in a liquid medium before and after growth of the fungus in order to determine whether the total quantity has been significantly reduced. Unfortunately, the chemical methods which are available for the determination of individual sugars, and particularly of polyhydric alcohols, although accurate when only the sugar or polyhydric alcohol in question is present, are not completely specific. Other compounds which are capable of reducing alkaline copper reagents or of being oxidized by potassium dichromate, are also estimated by these methods. Hence any reducing substances of this kind which may be formed during the growth of the fungus will be measured in addition to the particular carbohydrate being tested. Because of this source of error a lower utilization of the carbohydrate would be indicated than actually occurred.

If this limitation is borne in mind, however, the method of direct determination of carbohydrates before and after growth of the fungus should afford useful information as to their utilization.

The following experiments were based on these lines. The different strains of *Sporotricha* were grown in a basic liquid medium at a pH of 6.8 with and without the addition of various carbohydrates. At the end of four weeks the pH was again estimated, the weight of fungus growth was determined and the residual carbohydrate estimated by a titrimetric method. The weight of growth in the basic medium was subtracted from the weight in the carbohydrate medium, the difference being taken as an indication of the stimulatory effect of the carbohydrate. In the case of each carbohydrate a control uninoculated flask was left standing for the same length of time. Its pH and carbohydrate content were determined as accurately as possible. The difference in pH units between the pH of the control medium and those in which the fungi had grown was recorded. The difference between the carbohydrate content of the control and the culture media was taken as an

indication of the amount of carbohydrate utilized or altered by the fungus.

DETAILS OF TECHNIQUE

Basic medium

K_2HPO_4	1 gm.	
KCl	0.5 gm.	
$MgSO_4 \cdot 7H_2O$	0.5 gm.	
$FeSO_4$	0.018 gm.	
Sodium caseinate (Difco)	10 gm.	(Tested and found to be free from reducing substances)
Aq. dist.	1000 cc.	

A litre of water was heated to between 80 and 90° C and the inorganic salts added. The sodium caseinate was added slowly, during which time the mixture was stirred with a glass rod. When completely dissolved it was allowed to cool and was filtered through gauze. The volume was made up to 1000 cc. and the pH adjusted to 6.8; 50 cc. quantities were pipetted into each of eight 100 cc. Erlenmeyer flasks which were then autoclaved.

The carbohydrate medium was prepared in the same way except that the whole litre of medium was autoclaved, after which the carbohydrate was added to give a final concentration of 0.4 per cent. After dispensing 50 cc. quantities into sterile flasks, the flasks were steamed for one hour on three successive days.

The seven strains of fungus were maintained on Sabouraud's conservation agar containing no sugar. A platinum loop was drawn gently over the surface of the growth and a minimal amount of inoculum transferred to the respective liquid media. In each case a control flask was left uninoculated.

Whatman No. 42 filter papers were dried *in vacuo* over phosphorus pentoxide. Each filter paper was weighed in a weighing bottle, placed in an envelope and its weight recorded.

At the end of four weeks the content of each flask was filtered through these weighed filter papers using a Buchner funnel and slight suction. Each filter paper was then placed in a Petri dish and again dried and weighed. The difference in weight was recorded as the weight of growth.

The pH of the filtrate was estimated with a Beckman pH meter. The amount of carbohydrate present in each filtrate was determined titrimetrically. By subtracting the amount of carbohydrate in the various culture filtrates from that in the control, the amount of carbohydrate utilized or altered by the fungi was derived.

For each strain the weight of dry fungus produced by the basic medium was subtracted from that given by the carbohydrate medium. This gave some indication of the increase in growth due to the presence of the carbohydrate.

TECHNIQUE OF CARBOHYDRATE DETERMINATIONS

Monosaccharides (Modification of Benedict's method). An accurately measured 25 cc. of Benedict's reagent in a beaker is brought to boil. From a burette a standard 0.4 per cent solution of the particular carbohydrate in distilled water is added slowly until the blue color of the Benedict's reagent has almost disappeared. The mixture is boiled for three minutes until the reaction is complete. The carbohydrate solution is then added a few drops at a time, allowing ½ minute boiling after each addition. When the blue color is no longer visible a few drops of a 1 per cent solution of methylene blue is added to the mixture and the titration continued until all trace of blue has disappeared and a white precipitate remains.

After the Benedict's reagent is thus standardized, the procedure is repeated, titrating with the filtered medium. Usually, after a rough preliminary titration it was found necessary to use 10 cc. of Benedict's reagent and to dilute the medium 1 : 4 in order to obtain greater accuracy.

Disaccharides. To 25 cc. of a standard 0.4 per cent solution of the particular carbohydrate are added 15 cc. distilled water and 5 cc. $2N \cdot H_2SO_4$. The mixture is heated in a boiling water bath for five to ten minutes. After cooling, 5 cc. of $2N \cdot NaOH$ are added to neutralize the solution. The total volume is then made up to 100 cc. This hydrolyzed solution is titrated against Benedict's reagent in the same way as for monosaccharides. After the Benedict's reagent has been thus standardized the same procedure is applied to the filtered medium.

Polysaccharides. To 25 cc. of a standard 0.4 per cent solution of the particular carbohydrate are added 15 cc. distilled water and 15 cc. $2N \cdot H_2SO_4$. The mixture is heated in a boiling water bath for two and a half hours. From time to time a few drops of distilled water are added to replace that lost by evaporation. After cooling, 15 cc. $2N \cdot NaOH$ are added to neutralize the solution. The total volume is then made up to 100 cc. Thereafter the technique is the same as that for disaccharides.

Polyhydric alcohols. (Modification of A.O.A.C. method.) The following reagents are required:

1. Strong solution of $K_2Cr_2O_7$. 74.55 gm. pure dry $K_2Cr_2O_7$ dissolved in distilled water. Add 150 cc. conc. H_2SO_4, cool and dilute to 1 litre with distilled water.

2. Dilute solution of $K_2Cr_2O_7$. Dilute 50 cc. of the strong $K_2Cr_2O_7$ to 1 litre with distilled water.

3. Diphenylamine indicator. Dissolve 1 gm. diphenylamine in 100 cc. conc. H_2SO_4.

4. Retarder. Dilute 150 cc. of syrupy phosphoric acid with 600 cc. of distilled water and add 250 cc. conc. H_2SO_4.

5. Ferrous ammonium sulphate solution. Dissolve 30 gm. crystalline ferrous ammonium sulphate in distilled water, add 50 cc. conc. H_2SO_4, cool, dilute to 1 litre with distilled water.

Reagents 1, 2, 4 and 5 are diluted 1 : 3 before use.

To 25 cc. of the filtered medium are added 15 cc. of a 15 per cent trichloracetic acid solution and 10 cc. distilled water. The mixture is stirred, allowed to stand for 10 minutes and filtered through filter paper. To 25 cc. of this filtrate are added 30 cc. strong $K_2Cr_2O_7$ solution followed by 24 cc. conc. H_2SO_4. The mixture is heated in a boiling water bath for 20 minutes, cooled and diluted to 250 cc. with distilled water.

The ferrous ammonium sulphate solution is standardized by taking 20 cc. and then adding 20 cc. of the retarder, 4 drops of the indicator and 100 cc. distilled water, and titrating with the dilute $K_2Cr_2O_7$ solution until the liquid assumes a dark green color. The dilute $K_2Cr_2O_7$ solution is then added drop by drop until the color changes from blue grey to deep violet. (Volume of $K_2Cr_2O_7$ used = x cc.)

Twenty cc. of the ferrous ammonium sulphate (+ 20 cc. retarder + 4 drops indicator + 100 cc. distilled water) are next titrated with the oxidized medium. (Volume of solution used = y cc.)

The amount of polyhydric alcohol present in the 25 cc. sample taken for analysis is: $.01/3 \times (30 - 250x/20y)$ gm. The medium was originally diluted to half its strength with distilled water and trichloracetic acid. Therefore the amount of polyhydric alcohol in 25 cc. of the medium is $.01 \times 2/3 \times (30 - 250x/20y)$ gm.

The results of this experiment are summarized in table 3.

An examination of this table shows the following points:

1. In the basic medium without any carbohydrate the growth was very poor and the pH of the medium became more alkaline.

2. In the presence of glycerine, glucose, maltose, levulose and saccharose with all seven strains the medium became more acid, the weight of growth was improved and the concentration of sugar in the medium lowered.

3. With lactose and starch the changes in pH varied with the different strains, the growth weight of all strains was increased and the concentration of the carbohydrate lowered. There does not appear to be any relationship between the change in pH, the stimulation of growth and the amount of carbohydrate utilized, *e.g.*, in the case of lactose with strains No. 21 and 61, the reaction became more alkaline, there was no significant increase in growth but the concentration of carbohydrate was definitely reduced. With strains No. 13 and 32 there was no change in pH, a moderate increase in growth and a lowering in the carbohydrate content. One strain, No. 20, acidified the medium; its growth stimulation was less than that of No. 13 and 32 but the carbohydrate was lowered to the same extent.

4. In the presence of arabinose the medium became more alkaline with all strains, the growth weight was slightly increased but the sugar concentration was definitely reduced.

5. In the case of inulin the pH with all strains except No. 72 became more acid, the growth weight was increased but the carbohydrate was insignificantly reduced (except with strains No. 21 and 32).

6. With dextrine the pH became more acid; the growth weight of strains No. 8, 20, 32 and 72 was not significantly increased, that of the other strains being only slightly increased; an insignificant reduction in the carbohydrate occurred with all strains.

7. With glycogen the pH response varied with the different strains, there was no appreciable increase in weight but the carbohydrate content was slightly lowered.

8. In the presence of mannite the pH response was variable, no increase in weight was detectable and there was an apparent increase in the carbohydrate content.

In order to determine whether any reducing substance was present in the catabolic products, the seven strains were grown in the same basic medium without any carbohydrate. At the end of four weeks the filtered media were subjected to the potassium dichromate technique. It was found that with several strains a reducing substance had appeared in the medium. As this technique is incapable of differentiating mannite from other reducing substances the figures given in table 3 represent the total amount of reducing substance present. It is not possible, therefore, to state whether the concentration of mannite had been reduced or not. It can, however, be seen that there was no improvement in the growth of the fungus. The probability is, therefore, that mannite was not fermented.

In the case of glycerine, the figures given must be regarded as approximate. The total quantity of glycerine utilized probably exceeds the estimated amounts.

If one can accept the production of acid, the increased growth and the reduction of the carbohydrate content as evidence that the carbohydrate has been fermented, then all seven strains fermented glycerine, glucose, maltose, levulose and saccharose. In the case of arabinose, although the pH with all strains became more alkaline, there does appear to have been a utilization of the sugar. With lactose, inulin, dextrine, starch and glycogen the results were variable and inconclusive. It must, however, be borne in mind that there are limitations to the accuracy of the techniques employed for the determination of these carbohydrates, especially since the amounts utilized were small relative to the amounts added

TABLE 3

CORRELATION OF CHANGE IN pH AND WEIGHT OF GROWTH WITH UTILIZATION OF VARIOUS CARBOHYDRATES. (50 CC. SYNTHETIC LIQUID MEDIUM + 0.4% CARBOHYDRATE. ORIGINAL pH 6.8. FOUR WEEKS GROWTH AT ROOM TEMPERATURE)

Strain	Basic Medium							
	pH series A	pH series B	pH series C	Average pH	Weight of growth in mgms. Series A	Weight of growth in mgms. Series B	Weight of growth in mgms. Series C	Average weight of growth in mgms.
Control								
No. 8	7.32	7.74	7.9	7.65	8	5.5	3.5	6
No. 13	7.32	7.8	7.9	7.67	8	3.5	3.5	5
No. 20	7.25	7.7	7.55	7.5	10	5.5	7.5	8
No. 21	7.18	7.9	8.15	7.74	9	3.5	7	6.5
No. 32	7.18	7.75	7.95	7.63	9	9	4	8
No. 61	7.2	7.85	7.95	7.67	12	7	6	8
No. 72	7.32	7.7	7.9	7.64	12	6.5	6.5	8

Strain	Glycerine					Glucose				
	pH after 4 weeks	Difference between pH of culture and pH of control	Weight of growth in mgms.	Difference between weights of growth in carbohydrate and basic media	Difference between carbohydrate content of culture and control in mgms.	pH after 4 weeks	Difference between pH of culture and pH of control	Weight of growth in mgms.	Difference between weights of growth in carbohydrate and basic media	Difference between carbohydrate content of culture and control in mgms.
Control	6.52					5.7				
No. 8	5.8	−.72	35	29	−26	5.53	−.17	28.5	22.5	−150
No. 13	5.95	−.57	38.5	33.5	−26	5.42	−.28	33	28	−110
No. 20	5.8	−.72	40.5	32.5	−36	5.5	−.2	32	24	−120
No. 21	6.12	−.4	31.5	25	−72	5.5	−.2	28	21.5	−130
No. 32	6.15	−.37	40	32	−26	5.5	−.2	28.5	20.5	−165
No. 61	6.15	−.37	41	33	−26	5.52	−.18	35	27	−180
No. 72	6.0	−.52	38.5	30.5	0	5.5	−.2	27	19	−150

Strain	Mannite					Laevulose				
Control	7.23					6.26				
No. 8	7.3	+.07	11	5	+62	5.78	−.48	24	18	−130
No. 13	7.3	+.07	12	7	+28	5.6	−.66	34	29	−111
No. 20	7.2	−.03	13	5	+15	5.62	−.64	32	24	−160
No. 21	7.13	−.1	16.5	10	+42	5.63	−.63	28	21.5	−149
No. 32	6.99	−.24	13	5	+92	5.78	−.46	25	17	−111
No. 61	7.23	0	6.5	—	+18	5.75	−.51	27	19	−130
No. 72	7.13	−.1	18	10	+64	5.73	−.53	32	24	−149

TABLE 3—*Continued*

Strain	Dextrine					Inulin				
	pH after 4 weeks	Difference between pH of culture and pH of control	Weight of growth in mgms.	Difference between weights of growth in carbohydrate and basic media	Difference between carbohydrate content of culture and control in mgms.	pH after 4 weeks	Difference between pH of culture and pH of control	Weight of growth in mgms.	Difference between weights of growth in carbohydrate and basic media	Difference between carbohydrate content of culture and control in mgms.
Control	6.79					6.6				
No. 8	6.79	0	19	13	−10	6.11	−.49	35	29	− 6
No. 13	6.64	−.15	26.5	21.5	−15	6.15	−.45	37	32	− 2
No. 20	6.79	0	17.5	9.5	−10	6.15	−.45	42.5	24.5	− 2
No. 21	6.03	−.76	32	25.5	−12	6.12	−.48	31	24.5	−35.5
No. 32	6.29	−.5	21.5	13.5	− 9	6.35	−.25	33	25	−37.5
No. 61	6.32	−.47	31	23	− 4	6.4	−.2	44	36	−21
No. 72	6.21	−.58	22.5	14.5	− 5	7.4	+.8	23.5	15.5	−12
	Arabinose					Maltose				
Control	6.42					6.8				
No. 8	6.65	+.23	23	17	− 64	6.5	−.3	40	34	−40
No. 13	6.6	+.18	24	19	− 64	6.25	−.55	51	46	−56
No. 20	6.46	+.04	26	18	−145	6.75	−.05	48	40	−61
No. 21	6.54	+.12	21.5	15	− 72	6.05	−.75	40	33.5	−57
No. 32	6.48	+.06	27.5	19.5	−124	6.5	−.3	40	32	−78
No. 61	6.48	+.06	28	20	−124	6.5	−.3	44	36	−75
No. 72	6.6	+.18	26	18	−115	6.0	−.8	75	67	−79
	Lactose					Saccharose				
Control	7.0					7.3				
No. 8	7.15	+.15	21	15	−37	7.0	−.3	27	21	− 60
No. 13	7.0	0	33	28	−46	6.96	−.34	32.5	27.5	− 32
No. 20	6.85	−.15	25	17	−62	7.1	−.2	24	16	− 39
No. 21	7.3	+.3	15	8.5	−57	7.3	0	27.5	21	− 44
No. 32	7.0	0	37	29	−62	6.93	−.37	22.5	14.5	− 55
No. 61	7.15	+.15	19	11	−70	6.8	−.5	29	21	−125
No. 72	7.05	+.05	26	18	−59	7.28	−.02	30	22	− 46
	Starch					Glycogen				
Control	6.9					7.0				
No. 8	7.16	+.26	21.5	15.5	− 8	7.3	+.3	5.5	—	−14
No. 13	6.5	−.4	26	21	−20	6.63	−.27	20	15	−28
No. 20	7.12	+.22	23	15	−16	7.26	+.26	8	—	−33.5
No. 21	6.6	−.3	27.5	21	−19	6.73	−.27	13.5	7	−32
No. 32	6.6	−.3	26	18	−20	6.78	−.22	19.5	11.5	−17.5
No. 61	6.5	−.4	27	19	−36	6.78	−.22	16	8	−38
No. 72	6.0	−.9	25.5	17.5	−36	6.25	−.25	18	10	−34.5

to the medium. Lactose and starch are probably fermented to a slight extent, and possibly also dextrine, inulin and glycogen. It is almost certain that no strains ferment mannite. There is no clear-cut difference between the seven strains as regards their ability to ferment the carbohydrates.

While not all the carbohydrates were used in each of the three experiments, several interesting comparisons are possible.

1. Glycerine, glucose and levulose, according to experiment 3, appear to be fermented by all seven strains. In the two previous experiments on solid media much acid was produced with these three carbohydrates.

2. While no fermentation of mannite was demonstrable in experiment 3, acid was produced in both previous experiments.

3. In the first experiment only two strains showed the production of acid with saccharose and maltose. Yet in the third experiment all strains lowered the pH and appeared to ferment these sugars.

4. On solid media no strain produced acid with starch, yet in the liquid medium five strains produced an appreciable lowering of the pH.

DISCUSSION

In table 4 is summarized the available literature on the carbohydrate reactions of different species of *Sporotrichum*. It is clear that there are many conflicting results. However, most investigators, including this study, agree that pathogenic *Sporotricha* produce acid with glycerine, glucose, maltose and levulose. All except Blanchetière and Gougerot (**2**) agree that lactose is not fermented. They state that the original *Schenckii* strain definitely ferments lactose and that this is one of the important features in the differentiation of *S. Schenckii* from *S. Beurmanni.* Their statement has never been confirmed. Although the results of this study cannot definitely confirm or contradict this, no significant differences were shown by the *Schenckii* and *Beurmanni* strains. Blanchetiere and Gougerot (**2**) also maintain that *S. Beurmanni* ferments saccharose and that *S. Schenckii* does not. Most workers have confirmed this statement. In the present study there does

TABLE 4

SUMMARY OF AVAILABLE LITERATURE ON CARBOHYDRATE REACTIONS OF SPOROTRICHA

Author	Species	Glycerine	Glucose	Mannite	Lactose	Saccharose	Maltose	Laevulose	Galactose	Arabinose	Inulin	Dextrine	Starch	Dulcite	Adonite	Salicin	Xylose	Raffinose
Blanchetière and Gougerot (2)	Beurmanni	+	+	+	−	+	+		+		+	−		+				
Dodge (6)	Schenckii var. Beurmanni	+	+	−	−	?+	+				+	−	+	−				
Moore and Kile (13)	Beurmanni		+	−	−	+	+	+	+	−		+					+	
Walker and Ritchie (18)	? Beurmanni		+	+	−	+	+		+		+			−	−	−		+
Pijper and Pullinger (15)	Beurmanni		−	−	−	−	−	−	−	−	−	−		−	−			−
Panja, Dey and Ghose (14)	Beurmanni	+		−	−	+	+				+	−						
Ghose (7)	Beurmanni var. indicum		−	−	−	−	−	−		−	−			−		−		
Schenck (16)	Schenckii		−		−	−												
Blanchetière and Gougerot (2)	Schenckii				+	−												
Blanchetière and Gougerot, quoted by Wolbach, Sisson and Meier (19)	Schenckii	+	+		+						+							
Anderson and Spector (1)	? Schenckii		−	−	−	+	−											
Taylor (17)	? Schenckii		+	−	−	−	−											
Boggs and Fried (3)	? Schenckii		+	−	−	−	+											
Meyer and Aird (12)	Schenckii and Beurmanni	±	+	−	−	+ or −	+ or −	+	+									

TABLE 4—*Continued*

Author		Species	Glycerine	Glucose	Mannite	Lactose	Saccharose	Maltose	Laevulose	Galactose	Arabinose	Inulin	Dextrine	Starch	Dulcite	Adonite	Salicin	Xylose	Raffinose
Wolbach Sisson and Meier (19)		Councilmani	+	+	−	−	−					−			+				
Blanchetière and Gougerot, quoted by Wolbach, Sisson and Meier (19)		Gougeroti	+	+	−	−	+												
Dodge (6)		Grigsbyi		−		−	−												
Dodge (6) *M*		Fonsecai	+					+	+										
Panja, Dey and Ghose (14)		Tropicale	+	+	+ late	−	−	+	+	+	−	−	+			−		+	−
Blanchetière and Gougerot, quoted by Meyer and Aird (12)		All pathogenic	+	+	−	−	+	+	+	+				+					
This study	Experiment No. 1	Various species	+	+	+	−	+ or −	+ or −						−					
	Experiment No. 2	Various species	+	+	+				+		−	+ or −	+ or −		−	+ or −			
	Experiment No. 3	Various species	+	+	+ or −	+ or −	+	+	+		−	+	+	+ or −					

+ = acid. − = no acid.

not appear to be any difference between the two species. *S. Councilmani, Grigsbyi* and *tropicale* are said not to ferment saccharose, whereas *S. Gougeroti* does do so. In this study it was found that *S. tropicale* ferments this sugar.

With regard to mannite all except two investigators agree that this carbohydrate is not fermented. *S. tropicale* is said to be a late fermenter. None of the species investigated in this study, including *S. tropicale,* fermented mannite after twenty-eight days.

Carbohydrates other than those mentioned above have not yet been adequately investigated.

It is more than likely that the discrepancies are due to differences in technique and in the interpretation of what constitutes evidence of fermentation. The production of acid alone is obviously insufficient evidence. It is worthy of note at this stage that Dodge (**6**) says of *S. Fonsecai:* "No fermentation of sugars; acid with glucose, fructose and maltose."

CONCLUSION

It would appear that pathogenic *Sporotricha* ferment glycerine, glucose, maltose and levulose, but probably do not ferment mannite or lactose to an appreciable extent. With regard to the other carbohydrates information is as yet inadequate. It appears to be extremely unlikely that the carbohydrate reactions will prove to be of any value in the differentiation of species.

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* Not read in the original.

NEW SPECIES OF CELLULOSE DECOMPOSING FUNGI. II

L. M. Ames *

(with 13 figures)

The present contribution deals with fungi isolated either from military materiel and equipment or from other sources. However, the three species herein described are of importance, both from military and civilian viewpoints, because of their cellulose destroying characteristics. These species have been cultivated on maize meal and potato extract agar with a strip of cloth or filter paper added, with 2% agar-agar, as described in a previous paper [1] to insure uniformity of growth conditions for all species of the Chaetomiaceae that have been described recently or that will be described in my future work. Exceptions, when necessary, will be explicitly reported.

The writer thanks the following men for their kindness in sending the material which is the subject of this paper: Dr. G. W. Martin, University of Iowa; Dr. E. A. Bessey, Michigan State College; and Dr. J. C. Gilman, Iowa State College.

Chaetomium fibripilium sp. nov.

Viridoflavum vel gilvo-brunneum. Peritheciis mediae vel magnae magnitudinis, subglobosis vel ovatis, basi acuminatis, $260 \times 230\,\mu$ ($200–330 \times 185–285\,\mu$) ostiolatis, cum rhizoideis gracilibus paratis, interdum cum cirrhis. Pilis lateralibus numerosis, gilvo-viridantibus cum senectute gilvo-brunneis basi rectis, apicibus undulatis abnormis, septatis, basi $2.25–3.25\,\mu$ diametro, apicibus acuminatis. Pilis terminalibus numerosis, intertexis, gilvo-viridantibus, cum senectute gilvo-brunneis, simpliciter vel compositer ramosis, septatis, capitem frutectosum magnum formans, basi $2.25–3.5\,\mu$ diametro, apicibus undulatis abnormis et acuminatis. Ascis clavatis, octosporis, fugacibus. Ascosporis maturis dilute brunneis, ovatis, in ultima subapiculatis, $10 \times 8\,\mu$ ($9.5–11.25 \times 7–8.5\,\mu$).

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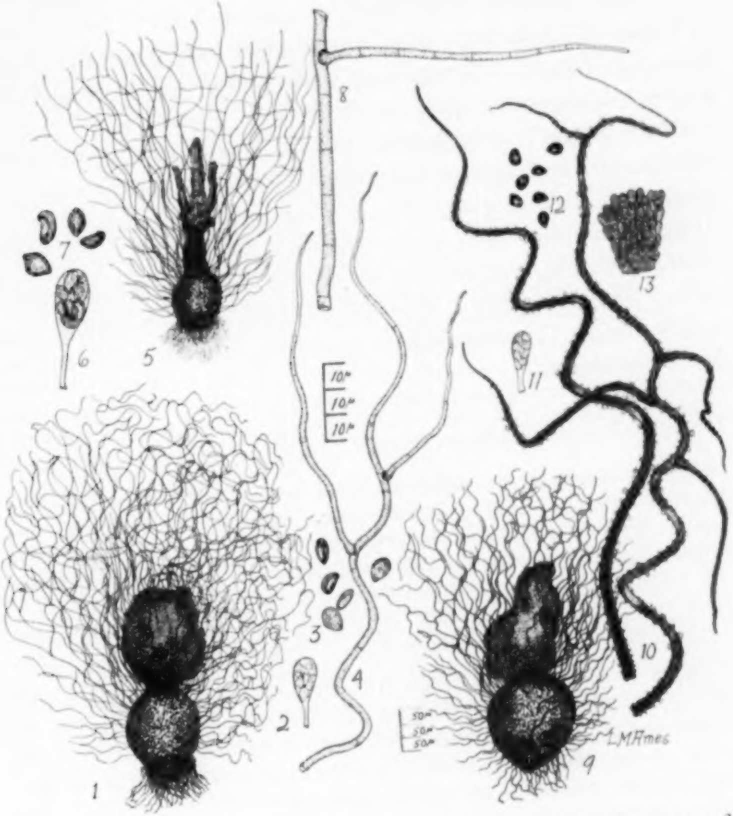
[1] Ames, L. M. New cellulose destroying fungi isolated from military material and equipment. Mycologia **41**: 637–648. 1949.

Greenish yellow to yellowish brown. Perithecia medium to moderately large in size, subglobose or ovate with bluntly pointed bases $260 \times 230\ \mu$ ($200–330 \times 185–285\ \mu$), ostiolate, supported by delicate rhizoids, occasionally producing cirrhi. Lateral hairs numerous, greenish yellow, browning with age, straight near base, irregularly wavy toward the apex, distinctly septate, at base $2.25–3.25\ \mu$



FIGS. 1–4, *C. fibripilium:* 1, mature perithecium; 2, immature ascus; 3, mature ascospores; 4, branched terminal hair. FIGS. 5–8, *C. mollipilium:* 5, mature perithecium; 6, mature ascus; 7, mature ascospores; 8, detail of branched terminal hair. FIGS. 9–13, *C. nigricolor:* 9, mature perithecium; 10, detail of two terminal hairs, one unbranched, the other branched; 11, immature ascus; 12, mature ascospores; 13, small group of young asci as seen when the perithecium is crushed, almost transparent in water mounts, this shown after staining.

in diameter, gradually tapering to the apex. Terminal hairs numerous, interwoven, greenish yellow when young, browning with age, unbranched or branched, some compositely branched, distinctly septate, forming a large bushy head, at base 2.25–3.5 μ in diameter gradually tapering to the apex. Asci club-shaped, 8-spored, fugaceous. Ascospores dilute green when young, when mature dilute brown, ovate, slightly apiculate at one end, round at the other, 10 × 8μ (9.5–11.25 × 7–8.5 μ).

Type. Isolated from sugar cane which was collected in the Hawaiian Islands. Culture was made by Dr. E. A. Bessey and sent to the writer for study.

C. fibripilium is distinguished, when young, by its neutral green ascospores which become pale brown with age, and its compact head of hairs, many of which are branched compositely. The branched terminal hairs are quite similar in structure to those of *C. sphaerale* Chivers, but differ in color, *C. sphaerale* being grayish-yellow to olive-yellow. The two species differ regarding the ostiole region, *C. sphaerale* having a distinct neck which is lacking in *C. fibripilium*.

Chaetomium mollipilium sp. nov.

Fulvum. Peritheciis magnitudinis mediae, globosis vel subglobosis, 175 × 130 μ (165–240 × 130–190 μ) ostiolatis, cum rhizoideis fulvis paratis, cum cirrhis. Pilis lateralibus, paucis, gracilibus, septatis, basi 3–3.75 μ, apicibus acuminatis. Pilis terminalibus moderate longis, gracilibus, septatis, basi 3.25–4.25 μ diametro, saepe ramosis, divaricatis, apicibus acuminatis. Ascis clavatis, octosporis, 38 × 12.5 μ, parte sporifera 27 μ. Ascosporis maturis fulvis, limoniformibus, utrinque apiculatis, 10–12.5 × 8.5–10 μ.

Light brown to cream-colored. Perithecia of medium size, globose to subglobose 175 × 130 μ (165–240 × 130–190 μ,), ostiolate, supported by light brown rhizoids, with cirrhi. Lateral hairs moderate in number, wavy, seldom branched, septate, graceful, at base 3–3.75 μ in diameter, gradually tapering to the apex. Terminal hairs sparse, forming a relatively open head, septate, often branched with wide angles, at base 3.25–4.25 μ in diameter, gradually tapering to a narrow tip. Asci clavate, eight-spored, 38 × 12.5 μ, spore part lemon-shaped, apiculate at both ends, 12 × 10 μ (10–12.25 × 8.5–10 μ).

Type. Isolated from a Japanese raincoat. Culture was made by Dr. G. W. Martin while he was at the Jeffersonville Quarter-

master Depot, Jeffersonville, Indiana, and designated as J–587, APO, 565. A similar isolate was designated as J–905.

This species is distinguished by its soft, easily mashed hairs, many of which are branched with wide angles. Looking directly down on comparatively fresh cultures the outline of the perithecium is clearly visible due to the paucity of terminal hairs. This species has been isolated four times from diseased fingernails. Nail specimens from four sources were washed in 95 per cent alcohol, rinsed in sterile distilled water and then placed on mineral agar. The perithecia which developed were found growing on the nail specimens. This is reported only as an interesting observation of apparent human nail infection by a species of *Chaetomium*.

Chaetomium nigricolor sp. nov.

Nigrum. Peritheciis magnitudinis mediae, globosis vel subglobosis, 250 × 230 μ (200–285 × 190–260 μ) ostiolatis, cum rhizoideis brunneis paratis, interdum cum cirrhis. Pilis lateralibus paucis gracilibus, obscure septatis, apicibus acuminatis, diametro 3.5–4.5 μ, scabris. Pilis terminalibus longis, numerosis, moderate rigidibus, scabridis, obscure septatis, compositer vel simpliciter ramosis, basi 3–4 μ diametro, apicibus acuminatis. Ascis clavatis, octosporis, 25 × 9 μ, parte sporifera 14 μ. Ascosporis maturis brunneis, amigdalo-formibus, apice subapiculatis, 5–6 × 4–5 μ.

Black. Perithecia of moderate size, globose to subglobose, 250 × 230 μ (200–285 × 190–260 μ), ostiolate, attached to the substratum with brown rhizoids, seldom provided with cirrhi. The bushy haired heads have a ragged appearance. Lateral hairs moderately numerous, straight or undulating, obscurely septate, 3.5–4.5 μ in diameter, roughened, black in color and tapering to faded tips. Terminal hairs numerous, black, regularly and thickly covered with little black projections, with larger crystals or projections appearing at random, undulating or with occasional spiral coils, frequently branching, at base 3–4 μ in diameter, tapering to faded tips. Asci fugaceous, club-shaped, 8-spored, 25 × 9 μ, spore part 14 μ. Mature ascospores brown, almond-shaped, slightly apiculate at one end, 5–6 × 4–5 μ.

Type. From India. This species was received from Dr. J. C. Gilman, who received the specimen from India under the binomial *Chaetomium convolutum* Chivers. They are similar only in color. *C. nigricolor* is recognized by the black, intermingling branched and simple undulating terminal hairs, and the small, brown ascospores.

STUDIES IN THE GENUS CINTRACTIA. II. C. AXICOLA AND RELATED SPECIES

Lee Ling

(with 1 figure)

Cintractia axicola (Berk.) Cornu, as the type of the genus, is one of the most widely distributed and best known species. Very little confusion has occurred concerning it in recent literature. However, a related species occurring on *Cyperus, Cintractia limitata* Clint., has occasioned much confusion. In the present paper, descriptions and synonymy of five species of *Cintractia* infecting *Fimbristylis* and *Cyperus* are presented. Besides the names included here, two other species, *Cintractia tangensis* P. Henn. and *Cintractia javanica* Racib., have been recorded on *Cyperus*. The former has not been available for study and hence has been excluded. The host of the latter was found to be a *Rhynchospora*.

As in the previous series, the institutions in which the specimens examined are located are indicated by abbreviations. Abbreviations, which were not given in the previous paper, are as follows: BR = Jardin Botanique de l'Etat, Brussels; CMI = Commonwealth Mycological Institute, Kew; LP = Instituto de Botanica "Spegazzini," Universidad Nacional de La Plata; ND = Indian Agricultural Research Institute, New Delhi. Wherever the location is not given, the specimen is in the writer's personal collection. The writer is very grateful to those who furnished the material and facilities for study in their institutions; and to Mr. J. A. Stevenson for the review of the manuscript.

Cintractia axicola (Berk.) Cornu, Ann. Sci. Nat. Bot. VI. **15**: 279. 1883

Ustilago axicola Berk., Ann. Mag. Nat. Hist. II. **9**: 200. 1852.
Ustilago fimbristylis Thuem., Bull. Torrey Bot. Club **6**: 95. 1876.
Ustilago peribebuyensis Speg., Anal. Soc. Cien. Argent. **17**: 89. 1884.
Cintractia peribebuyensis Speg., Anal. Soc. Cien. Argent. **26**: 11. 1888.

Cintractia fimbristylis-kagiensis Saw., Dept. Agr. Gov't Res. Inst. Formosa Rep. **2**: 79. 1922.

Cintractia peribebuyensis Saw., Dept. Agr. Gov't Res. Inst. Formosa Rep. **2**: 80. 1922.

Cintractia suedae Saw., Dept. Agr. Gov't Res. Inst. Formosa Rep. **2**: 81. 1922.

Cintractia mundkuri Chowdhury, Indian Jour. Agr. Sci. **14**: 231. 1944.

Cintractia peribebuyensis Speg. var. *major* Pavgi & Mundk. Indian Phytopath. **1**: 109. 1948.

Sori at the base of primary or auxiliary rays, less often in the ovaries, globoid to oblong, 1–4 mm. in length, at first each covered by a whitish false membrane which soon disappears exposing the black, agglutinate, compact spore mass. Spores when mature medium to deep reddish brown, often with smoky tint, globose to oval, occasionally oblong or subangular, sometimes laterally compressed, smooth to indistinctly pitted, or faintly ornamented with short, fine, sinuous striae, 12–17 × 9–15.5 μ, epispore 1–1.5 μ thick.

Material examined: On *Fimbristylis autumnalis* (L.) R. & S. United States: Beaumont, Alabama, in Herb. Berk. 4745, sub *U. axicola* var. (K); Spiro, Oklahoma, in Barthol. Fungi Columb. 4406 (BPI); Virginia, type of *U. fimbristylis* (CH).

On *Fimbristylis diphylla* (Retz.) Vahl. Brazil: Apiahy, Aug. 1888, J. I. Puiggari 2875 (LP); Taquari, Mar. 1940, Costa Nets (LP). Ceylon: Bandarawela, Apr. 2, 1904, T. Petch 2552 (BPI). China: Canton, Kwangtung, C. W. Howard (BPI); Tsingyen ,Kweichow, Aug. 19, 1940, S. C. Liu; Chengtu, Szechwan, Nov. 1947, K. R. Lin; Taipeh, Taiwan, Nov. 12, 1920, H. Sueda, type of *C. suedae*. Dominican Republic: St. Domingo, in Herb. Berk 4745, *type* (K). Indo-China: Tonkin, Feb. 1889, B. Balansa 33, sub *Cintractia junci* Trel. (FH). Paraguay: Cordillera de Peribebuy, Mar. 25, 1883, B. Balansa 3775, type of *U. peribebuyensis* (LP).

On *Fimbristylis ? ferruginea* (L.) Vahl. Philippine Islands: Manila, Luzon, Sept. 30, 1945, C. T. Rogerson 687 (BPI).

On *Fimbristylis kagiensis* Hay. China: Taichung, Taiwan, Oct. 11, 1913, Y. Fujikuro, type of *C. fimbristylis-kagiensis*.

On *Fimbristylis monostachaya* (L.) Hassk. Philippine Islands: Manila, Luzon, Sept. 30, 1945, C. T. Rogerson 686 (BPI).

On *Fimbristylis* sp. Cuba: in C. Wright, Fungi Cub. Wright. 604 (FH). Paraguay: in Balansa, Pl. Parag. 4294, sub *Ustilago*

juncicola Speg. (FH). India: Cocanada, Madras, Dec. 1906, S. Sundararaman, type of *C. peribebuyensis* var. *major* (ND).

On *Fimbristylis tenera* R. & S. India: Pak Pattan, Punjab, Oct. 1936, S. Ahmad 78 (BPI).

In the type collection of this species, the spores are smooth; whereas in certain other collections they may be faintly ornamented by pittings or short striae. On the basis of this character alone, it seems not advisable to split this old and well-known species.

CINTRACTIA CYPERI Clint., Proc. Bost. Nat. Hist. **31**: 400. 1904

Sori in the ovaries, globoid, 1–1.5 mm. diam., more or less exposed between the spreading glumes at later stages; spore mass black, agglutinate and rather compact until maturity. Spores often loosely agglutinated into balls, chiefly polyhedral, 11–21 × 9–16 μ, most elongate ones up to 25.5 μ in length, yellowish brown; epispore 1.5 μ thick, under oil immersion lens appearing as if decorated by very short, sinuous, depressed striae.

Material examined: On *Cyperus filiculmis* Vahl. United States: North Haven, Connecticut, in Seym. & Earle, Econ. Fungi C102, *type* (BPI); Montowese, Connecticut, Sept. 14, 1903, G. P. Clinton (CH); Ocean Gate, New Jersey, Aug. 17, 1919, P. Wilson (BPI).

Zundel (**3**) recorded *Cyperus cylindricus* and *C. grayii* as the hosts of this species. The latter name is apparently a mistake, since the New Jersey collection actually contains a mixture of healthy plants of *C. grayii* and smutted plants of *C. filiculmis*.

This species is closely related to *Cintractia gigantospora* Liro but differs from it in having chiefly polyhedral and lighter colored spores.

CINTRACTIA FIMBRISTYLICOLA Pavgi & Mundk., Indian Phytopath. **1**: 108. 1948

Sori in the ovaries, globoid, 1–1.5 mm. in diameter, black, compact, each at first covered by a thin white false membrane. Spores deep reddish brown, globose to oval, mostly laterally compressed, 8–12 μ in length; epispore approximately 1 μ thick, densely verruculose, often appearing as semi-reticulate under higher magnifications.

Material examined: On *Fimbristylis complanata* Link. India: Chatrapur, Ganjam, Orissa, Aug. 30, 1904, E. J. Butler, *type* (ND).

This species is very similar to *Cintractia fimbristylis-miliaceae* (P. Henn.) Ito and can be distinguished from the latter only by smaller and deeper colored spores. More collections should be studied to ascertain the range of variation in this group of fungi.

CINTRACTIA FIMBRISTYLIS-MILIACEAE (P. Henn.) S. Ito, Trans. Sapporo Nat. Hist. Soc. **14**: 92. 1935

Ustilago fimbristylis-miliaceae P. Henn., in Engl. Bot. Jahrb. **37**: 156. 1905.
Cintractia clintonii Cif., Ann. Myc. **26**: 30. 1928.
Cintractia pulchra S. Ito, Trans. Sapporo Nat. Hist. Soc. **14**: 92. 1935.

Sori in the ovaries, forming globoid, black, compact spore masses, 0.5–1.5 mm. in diameter, each covered at an early stage by a whitish false membrane. Spores when mature yellowish to reddish brown, spherical to oval, occasionally subangular, densely verruculose, 9.5–14.5 μ in length, epispore 0.5–1 μ thick.

Material examined: On *Fimbristylis holwayana* Fernald. Mexico: Guadalajara, Oct. 12, 1903, C. G. Pringle, type of *C. clintonii* (CH).

On *Fimbristylis miliacea* Vahl. Japan: Yukatabara, Iwaki, Kisuta, Sept. 1890, S. Kusano 782, *type* (CH); Mikazuki, Tosa, Oct. 1907, T. Yoshinaga, type of *C. pulchra;* Obi, Oct. 1890, S. Yoshida (FH).

The spores of this species are not smooth as originally described by Hennings. The Mexican collection differs from those from Japan in having a thicker, deeper colored, and less evidently verruculate epispore. The variation may be due to the degree of maturity. The Mexican collection is apparently in an earlier stage of development which is indicated by the presence of numerous, hyaline, agglutinate, immature spores.

CINTRACTIA LIMITATA Clint., Proc. Bost. Soc. Nat. Hist. **31**: 399. 1904. (FIG. 1)

Cintractia axicola (Berk.) Cornu var. *minor* Clint., Jour. Myc. **8**: 143. 1902.
Cintractia togoensis P. Henn., in Engl. Bot. Jahrb. **38**: 119. 1905.
Cintractia congensis P. Henn., Ann. Mus. Congo Bot. V. **2**: 87. 1907.

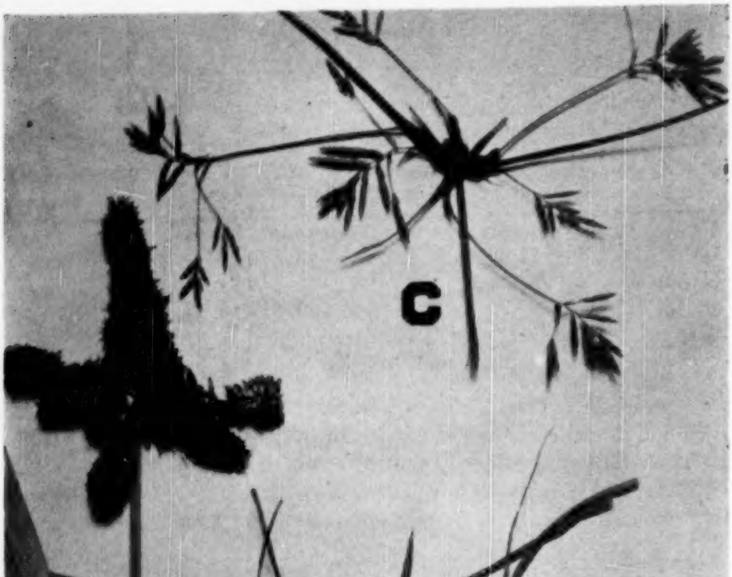


FIG. 1. *Cintractia limitata.*

Cintractia cyperi-polystachyi P. Henn., Philipp. Jour. Sci. C. **3**: 41. 1908.
Cintractia minor Jacks., Mycologia **12**: 153. 1920.
Ustilago chacoensis Hirschh., Not. Mus. La Plata **6**: 479. 1941.
Ustilago mariscana Zundel, Mycologia **35**: 165. 1943.
Cintractia distans Mundk., Indian Jour. Agr. Sci. **14**: 50. 1944.

Sori at the base of primary or auxiliary rays, cylindrical, forming black, agglutinate spore masses surrounding the branches, extending up to 1 cm. in length and 7 mm. in diameter, each covered at first by a whitish false membrane which soon disintegrates; often also infecting the ovaries, each forming a globoid black spore mass surrounding a rudimentary columella, 1–2 mm. in diameter, agglutinate at earlier stages, becoming rather dusty, exposed between the spreading glumes, and often spreading to adhere to the glumes and other floral parts. Spores when mature ochraceous brown to deep reddish brown, often with smoky tint, spherical to oval, occasionally subangular, frequently laterally compressed, $9–14.5 \times 7.5–13\ \mu$; epispore smooth, 1 μ or less thick.

Material examined: On *Cyperus compressus* L. China: Taitung, Taiwan, May 21, 1911, K. Sawada.

On *Cyperus corymbosus* Rottb. Ceylon: Peradeniya, June 29, 1913, T. Petch 3719.

On *Cyperus cyperoides* (L.) O. Ktze. Gold Coast: Abori, May 12, 1925, R. H. Bunting (CMI). Nigeria: J. West 25 (CMI). Sierra Leone: Newton, June 26, 1935, F. C. Deighton (CMI). Uganda: Serere Teso, June 29, 1939, C. G. Hansford 1602 (CMI). Union of South Africa: Imfulazane, Melmoth, Natal, Dec. 1, 1919, A. O. D. Mogg, type of *U. mariscana* (BPI).

On *Cyperus digitatus* Roxb. China: Taichung, Taiwan, July 2, 1936, Y. Hashioka.

On *Cyperus distans* L. f. Belgian Congo: Kinshasa, June 1, 1916, H. Vanderyst (BR); prov. Orientale, Apr. 1926, J. Ghesquière 562 (BR). India: Dacca, Bengal, Aug. 1940, P. Maheshwari, type of *C. distans* (ND).

On *Cyperus dubius* Rottb. Uganda: Serere Teso, June 29, 1939, C. G. Hansford 1644 (CMI).

On *Cyperus esculentus* L. Belgian Congo: Kelomila, May 1906, H. Vanderyst 105, type of *C. congensis* (BR); Boma, Apr. 1913, H. Vanderyst 1299 (BR).

On *Cyperus grayii* Torr. United States: Vineland, New Jersey, July 9, 1912, W. R. Jones (BPI); Sandy Hook, New York, in

Ell. & Ev. N. Amer. Fungi 2423, type of *C. axicola* var. *minor* (BPI).

On *Cyperus ligularis* L. Puerto Rico: Mayaguez, Apr. 23, 1904, G. P. Clinton, *type* (CH). Venezuela: Ocumare de la Costa, Mar. 19, 1938, C. E. Chardon 2489 (BPI).

On *Cyperus longus* L. Equatorial Africa: Gabun, June 1908, J. Mildbraed (CH).

On *Cyperus macrocarpus* (Kunth.) Boeck. var. *pseudoflavus* (K. Schum.) Kuekenth. Belgian Congo: Kinsatu, May 5, 1906, H. Vanderyst 32 (BR). West Africa: Lome, Togo, Camerun, Apr. 1900, Warnecke 118, type of *C. togoensis* (CH).

On *Cyperus malaccensis* Roxb. China: Taipeh, Taiwan, Sept. 27, 1917, Y. Fujikuro. Philippine Islands: Aparri, Cagayan, Luzon, Jan. 8, 1924, M. S. Clemens (BPI).

On *Cyperus ornans* Suring. Gold Coast: Anjinam, June 22, 1937, F. C. Deighton (CMI).

On *Cyperus polystachyus* Rottb. Philippine Islands: Manila, Aug. 1906, E. D. Merrill 5195, type of *C. cyperi-polystachyi*.

On *Cyperus rotundus* L. Anglo-Egyptian Sudan: Kadugli, Oct. 1947, J. Tarr (CMI). Argentina: Chaco, May, 1940, C. Carrera, type of *U. chacoensis* (LP).

On *Cyperus* sp. Brazil: Restinga de Cabo Frio, Rio de Janeiro, Oct. 16, 1938, A. P. Viégas & H. P. Krug (BPI).

On *Cyperus sphacelatus* Rottb. Nigeria: J. West 39 (CMI). Puerto Rico: Humacao Playa, H. H. Whetzel *et al.* 3038 (BPI); Manati, Jan. 25, 1938, W. A. McCubbin (BPI).

On *Cyperus tuberosus* Rottb. India: Lahore, Punjab, 1938, H. Chaudhari (CMI).

On *Cyperus zollingeri* Steud. Sierre Leone: Kichom, N. prov., June 1, 1928, F. C. Deighton (CMI).

The names commonly applied to this fungus are *C. peribebuyensis*, *C. minor* and *C. limitata*. As indicated in a previous note (**2**), the first is a synonym of *C. axicola*. The latter two were supposed to be distinguished by the location of sori, *C. minor* being confined to the base of the rays and *C. limitata* to the spikelets. However, even in the type collection of *C. minor* (FIG. 1, B), as mentioned by Clinton (**1**) himself, the infection also occurs in the floral organs forming a conspicuous smutty mass. It is also not uncommon to

find in the collections labelled as *C. limitata* that both the spikelets and the base of the rays are smutted by the same fungus. Figure 1 A, illustrates such a case in a collection from Venezuela on *Cyperus ligularis,* the type host of *C. limitata.* Therefore there is no real distinction between these two species in the characters of both the sori and the spores. According to the International Rules of Botanical Nomenclature, *C. limitata* is the valid name.

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2. **Ling, L.** Nota sinonimica sobre *Cintractia peribebuyensis.* Rev. Argent. Agron. **15**: 254–255. 1948.
3. **Zundel, G. L. I.** Ustilaginales. Additions and corrections. N. Amer. Fl. **7**: 971–1030. 1939.

EXPLANATION OF FIGURE

FIG. 1. *Cintractia limitata.* A. On *Cyperus ligularis,* showing infection in the spikelets and at the base of the ray (Venezuela, C. E. Chardon 2489). B. On *Cyperus grayii,* showing infection in the spikelets and at the base of the rays (type of *Cintractia axicola* var. *minor*). C. On *Cyperus polystachyus* (type of *Cintractia cyperi-polystachyi*). D. On *Cyperus macrocarpus* var. *pseudoflavus* (Congo, H. Vanderyst 32, sub *Cintractia togoensis*). All × 1.

A NOTE ON THE CULTURE OF DIPODASCUS UNINUCLEATUS IN DEFINED MEDIA *

EUGENE L. DULANEY AND F. H. GRUTTER

During a recent survey of fungi for the production of vitamins, it was found that *Dipodascus uninucleatus* failed to grow in certain media. Good growth, however, was obtained in a medium containing 5.0 grams of glucose and 10.0 grams of Difco yeast extract per liter of distilled water. When three-day old mycelium grown in this glucose-yeast extract broth was washed with sterile distilled water and used to inoculate a vitamin free medium, only sparse growth resulted. The mycelium constituting the sparse growth in the vitamin free medium was likewise washed and used to inoculate other flasks of the vitamin free medium. No visible growth occurred. The vitamin free medium, which will be referred to hereafter as the basal medium, contained the following constituents per liter of distilled water: glucose 10.0 g., vitamin free acid hydrolyzed casein 10.0 g., K_2HPO_4 2.0 g., $MgSO_4 \cdot 7H_2O$ 1.0 g., NaCl 0.2 g., $CaCl_2$ 0.2 g., $FeSO_4 \cdot 7H_2O$ 0.005 g. and $ZnSO_4 \cdot 7H_2O$ 0.005 g.

The results noted above indicate that *Dipodascus uninucleatus* requires one or more factors found in yeast extract for its growth. These possible nutritional deficiencies were investigated using the following experimental conditions. The media were dispensed in 25 ml. amounts in 125 ml. Erlenmeyer flasks which were plugged with non-absorbent cotton and sterilized by autoclaving at 120 lbs. for 17 minutes. All glassware was acid cleaned and thoroughly rinsed in distilled water before using. A master inoculum was prepared as follows and used for all experiments. Thoroughly washed mycelium grown in glucose-yeast extract broth was used to inoculate four flasks containing 25 ml. of the basal medium. After 72 hrs. incubation at 28° C on a rotary shaker, the sparse growth was removed from the flasks by centrifugation, pooled, washed three times with sterile distilled water and made up to a final vol-

* Contribution from the research laboratories of Merck & Co., Inc., Rahway, N. J.

ume of approximately 45 ml. with sterile distilled water. This washed suspension was stored at 4° C for several weeks and used at intervals. Two drops of this washed suspension were used to inoculate each flask. Each treatment was run in triplicate. All experiments were incubated at 28° C on rotary shakers moving at 220 r.p.m. so that they described a horizontal circle one and one-half inches in diameter.

It seemed probable that the required factors in the yeast extract were vitamins or nucleic acid constituents or both. This possibility was investigated by supplementing the basal medium with a vitamin mixture and nucleic acids singly and in combination. The results obtained after three days incubation are shown in table I.

TABLE I

A VITAMIN MIXTURE AND NUCLEIC ACIDS AS SUPPLEMENTS TO THE BASAL MEDIUM

Treatment	Dry wt. mg./ml.	pH
Basal Medium	No growth	
B.M. plus vitamin mixture [1]	5.86	3.4
B.M. plus nucleic acids [2]	0.66	3.6
B.M. plus vitamin mixture [1] and nucleic acids [2]	5.56	3.1

[1] One ml. of vitamin mixture was added to 24 ml. of the basal medium. The final concentration of the different vitamins in each flask was as follows: riboflavin 1.0 μg./ml., i-inositol 0.1 mg./ml., biotin 0.04 μg./ml., vitamin B_1 0.1 μg./ml., glutamine 5.0 μg./ml., pantothenic acid 1.0 μg./ml., vitamin B_{12} 0.001 μg./ml., nicotinic acid 10.0 μg./ml., pyridoxine 10 μg./ml., choline 5.0 μg./ml., para aminobenzoic acid 10 μg./ml., and folic acid 0.1 μg./ml.

[2] 12.5 mg. of yeast nucleic acid and 12.5 mg. of thymus nucleic acid were added directly to each flask.

No growth was obtained in the basal medium whereas good growth occurred in the medium supplemented with the vitamin mixture. The growth in the nucleic acid supplemented medium was probably due to vitamin contaminants in the nucleic acids. The addition of both nucleic acids and the vitamin mixture did not increase the amount of cell weight over that obtained in the medium supplemented with vitamins alone.

In order to determine what specific vitamins were required, the same vitamins that had been used in the mixture were divided into groups and used to supplement the basal medium. Growth occurred only in those treatments to which both vitamin B_1 and biotin were added. The effects of supplementing the basal medium

with biotin, vitamin B_1 and i-inositol singly and in combination are shown in table II. None of the vitamins alone stimulated growth.

TABLE II

VITAMINS USED SINGLY AND IN COMBINATION TO SUPPLEMENT THE BASAL MEDIUM

Treatment[1]	Dry wt. mg./ml.
Biotin	No growth
Vitamin B_1	No growth
I-inositol	No growth
Biotin and vitamin B_1	4.9
Vitamin B_1 and i-inositol	0.57
Biotin and i-inositol	No growth
Vitamin B_1, biotin, and i-inositol	5.1
Vitamin mixture	5.2

[1] One ml. of each vitamin solution was added to enough medium in each flask so that the final volume in each flask was 25 ml. This resulted in some slight variation in the concentration of medium constituents in those flasks to which more than one ml. of vitamin solution was added. The final concentration of each vitamin, however, was the same as the concentration of the same vitamin in the flasks containing the vitamin mixture.

There was some slight growth in the medium containing vitamin B_1 and i-inositol, and no growth in the medium containing biotin and i-inositol. Biotin and vitamin B_1, with or without i-inositol, replaced the vitamin mixture. No measurements have been made of the amount of growth that occurs in the glucose-yeast extract medium.

Dipodascus uninucleatus requires no exogenous supply of organic nitrogen. Three compounds were substituted for the hydrolyzed casein in an experiment, the results of which are shown in

TABLE III

SUBSTITUTION OF NITROGEN COMPOUNDS FOR THE CASEIN HYDROLYSATE

Treatment[1]	Dry wt. mg./ml.	pH
$(NH^4)_2HPO^4$	4.67	2.5
$NaNO^3$	No growth	
Urea	No growth	
Casein hydrolysate	4.43	3.1

[1] The casein hydrolysate was used at a concentration of 10 mg./ml. The other nitrogen compounds were used at a level of 5 mg./ml. All treatments contained 0.1 μg./ml. of vitamin B_1 and 0.04 μg./ml. of biotin.

table III. Urea and nitrate nitrogen did not serve as sole nitrogen sources. Ammonium nitrogen, however, was readily available.

These results indicate that *Dipodascus uninucleatus* requires an exogenous source of biotin and vitamin B_1. The possibility exists, however, that these deficiencies are peculiar to our strain and that, as a rule, the genus *Dipodascus* is not heterotrophic. Further investigation of other isolates is needed to establish this.

The drop in pH of the culture media in these experiments indicates the accumulation of one or more organic acids.

A NEW ACHLYA FROM FLORIDA [1]

A. W. ZIEGLER [2]

(WITH 15 FIGURES)

A water collection from a swamp in Wakulla County, Florida, 15 miles south of Tallahassee, Florida, has yielded an undescribed species of *Achlya*.

Achlya tuberculata n. sp.

Mycelium densum; hyphae usque ad 1 cm. longae in seminibus *Cannabis sativae;* cultura circa 1.5–2.5 cm. in diametrum. Sporangia copiosa in culturis juvenilibus, longa, cylindrica, 162–448 μ longa et 10–15 μ lata. Ejectio sporarum typica generis. Gemmae copiosae praesertim in culturis natu majoribus, valde variae. Oogonia globosa aut aliquando clavata, 44.4–92.5 μ in diametrum, ornata projectionibus obtusis, 1.2–3.7 μ longis; tunica tenuis in apice projectionis. Longitudo caulis oogonii 21.5–184 μ. Ova eccentrica, plerumque 3, globosa aut aliquando ovoidea, plurimum ferme 24.1 μ in diametrum, non complentes oogonium. Dimidia ovorum auctorum in aqua distillata deficit ante maturitatem; in decoctione foliorum *Quercus* ferme omnia ova maturescunt. Antheridia rara, semper diclina, reperta praecipue in culturis juvenilibus in aqua distillata aut cum aut sine foliis *Quercus.*

Mycelial growth dense, hyphae reaching a length of one-half to 1 cm. on hemp seed in charcoal filtered distilled water, diameter of the culture about 1.5–2.5 cm. Sporangia plentifully formed in young cultures, long, cylindrical, ranging from 162 by 10 μ to 448 by 15 μ. Spore discharge typical for the genus. The cluster of spores usually falls to the bottom of the dish soon after formation and gradually breaks apart into individual spores. Gemmae plentiful especially in older cultures, consisting of a row of swollen or non-swollen hyphal segments, single enlarged spherical or reniform bodies, or smaller spherical cells in a short chain. Gemmae germinate by a germ tube producing spores in the manner of the genus. Oogonia spherical or occasionally clavate with blunt pro-

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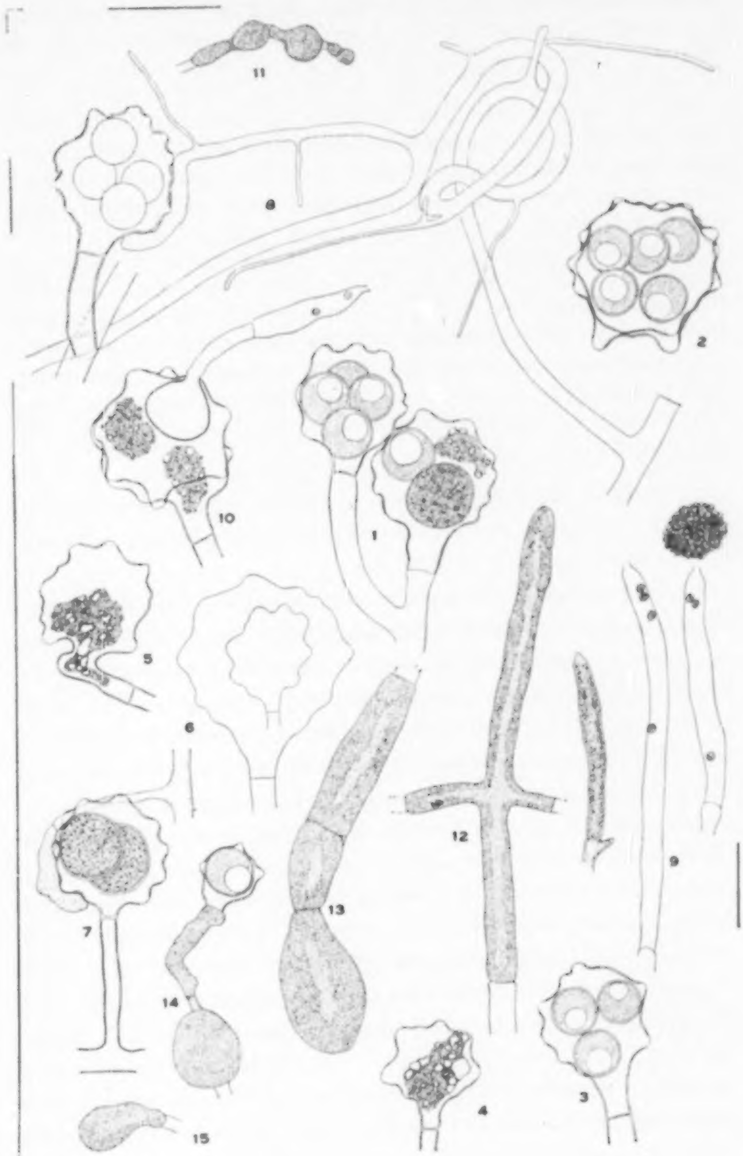

FIGS. 1–15. *Achlya tuberculata.*

jections and varying in size without the projections from 44.4 μ to 92.5 μ in diameter. The end wall of the projections is usually thinned. Projections vary from 1.2 μ to 3.7 μ in length. Length of oogonial stalk from 21.5 μ to 184.0 μ. Eggs eccentric, 1–6, mostly 3, ranging from 18.5 to 33.3 μ in diameter, mostly about 24.1 μ not filling the oogonium completely, usually spherical but sometimes ovoid through pressure. When grown in distilled water more than one-half of the eggs formed disintegrate before maturity even though an antheridium may be applied to the oogonial wall. However, if grown in distilled water to which oak leaves have been added 94–99% of the eggs reach maturity. Antheridia scantily formed and occurring mainly in young cultures in water with or without the addition of oak leaves. Antheridia always diclinous and those that appear to function become empty before the eggs are mature. Antheridial tube seen only twice. Mature eggs germinate after 2 months by short germ tubes with apical sporangia.

In water on hemp seed bait. Collected by the author in a swamp in Wakulla County, Florida, 15 miles south of Tallahassee, Florida, July 9, 1949.

On first seeing this fungus, the author classified it as *A. crenulata* Ziegler. However, later observations show that it differs in several ways. The oogonia of *A. tuberculata* show a tremendous size variation which is not characteristic of *A. crenulata*. The length of the oogonial stalk is highly variable in *A. tuberculata* whereas that of *A. crenulata* is about equal to the width of the oogonium. There is also a slight difference in the usual egg size between the two forms and the gemmae of *A. tuberculata* are wholly unlike those of *A. crenulata* (**4**).

One of the peculiar characteristics of this form is its disintegrating eggs which occur when it is grown in charcoal filtered distilled water in Petri dishes. The average of four separate counts of 100 oogonia each from four cultures of varying ages showed that 59% of the oogonia contained distintegrated eggs entirely. Of the remaining 41%, 28% of the oogonia contained eggs all of which developed to maturity, and 13% contained both mature and disintegrated eggs. No parasite has been found to be present and tests have been made which proved that the cultures were bacteria free. However, if the form is placed in an oak leaf decoction made by boiling a few dry oak leaves in distilled water for 10 minutes the

egg disintegration is considerably lessened. In this procedure, 94–99% of the oogonia formed mature eggs. Antheridial growth is only very slightly stimulated by this treatment. The characteristic discussed above is not a feature of the growth of *A. crenulata*. Experiments involving solutions of known pH and content, and the effect of returning the form to its natural habitat are now being performed by Miss Betty Linthicum and the author.

A. tuberculata may be distinguished from *A. subterranea* Coker and Braxton by the size of its oogonia and the usually androgynous antheridium of *A. subterranea* (**2**). It differs from *A. recurva* Cornu in the bluntness of its spines and in its diclinous antheridia (**3**) and from *A. radiosa* Maurizio especially in the number of eggs produced (**1**). The new form can easily be separated from *A. abortiva* Coker and Braxton by its spherical oogonia, pitted oogonial walls, and smaller eggs (**2**). The eccentric eggs and diclinous antheridia of *A. tuberculata* distinguish it from *A. colorata* Pringsheim (**1**).

It may be of interest to note that this fungus was collected in a water sample from a cypress swamp whose waters were exposed to direct sunlight all day. The author failed to take the temperature of the water but it felt like a very warm bath to the hand. *A. tuberculata* is grown in the laboratory on maltose peptone agar but is very slow in its growth rate.

Gemma formation apparently follows no set pattern in this species. The gemmae are often irregularly shaped and bizarre in form. A vegetative hypha often forms a row of swollen or non-swollen cells with the apical one either rounded or pointed. The cytoplasm of the oogonial stalk occasionally produces an elongated or spherical gemma (FIG. 14). Large spherical or kidney shaped gemmae are often seen as well as short chains of cells strung together like beads. Numerous variations of the above patterns also occur. However formed, they germinate by short germ tubes and spores.

A. tuberculata germinated readily when the mature eggs were about two months old. The eggs were placed in distilled water filtered through charcoal and completed their germination in about 32 hours. The details of the germination process closely follow those described for *A. flagellata* Coker. This species formed a

short germ tube with an apical sporangium and therefore falls into type A of the patterns of germinations found in the Saprolegniaceae (**5**).

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EXPLANATION OF FIGURES

FIG. 1. Two oogonia with mature and disintegrating eggs, × 750. FIGS. 2–3. Oogonia with mature eccentric eggs, × 750. FIGS. 4–5. Oogonia with disintegrated eggs, × 750. FIG. 6. Outline drawing of 2 oogonia showing great variation in size, × 750. FIG. 7. Oogonium with diclinous antheridium and immature eggs, × 750. FIG. 8. Outline drawing of oogonium with coiled diclinous antheridial branch, × 750. FIG. 9. Typical sporangia, × 350. FIG. 10. Oogonium containing several disintegrated eggs and one which has germinated and produced a germ tube with an apical sporangium, × 750. FIG. 11. A gemma, × 350. FIGS. 12–13. Gemmae, × 750. FIG. 14. Gemmae formed from the oogonial stalk, × 350. FIG. 15. Gemma, × 350.

A NEW RUST ON DESCHAMPSIA [1]

D. B. O. Savile [2]

Aecidium Graebnerianum P. Henn. was described from a specimen on *Habenaria dilatata* from Death Valley, Calif. The species has since been recorded on *H. borealis, H. bracteata, H. gracilis, H. hyperborea, H. leucostachys* and *Orchis aristata*, in California, Oregon, Washington, Montana, British Columbia and especially, Alaska (Arthur, 1), but it has apparently never been reported east of the Rocky Mts. In 1949, aecia apparently indistinguishable from it were found at two stations in Quebec. Mr. A. Payette collected a rust on *Habenaria psychodes* at Rivière Ouelle, on the south shore of the Gulf of St. Lawrence, about 70 miles below Quebec City. The aecia in this specimen (DAOM 23448), collected on 20 July, were largely spent, but sufficient spores were available to show that it agreed well with Barth. N. A. Ured. 1401, on *H. gracilis* from Glacier, B. C.

On the same date that Mr. Payette made his collection, the writer found a similar *Aecidium* at Great Whale River, on the east coast of Hudson Bay, on *H. dilatata* (DAOM 23447). Owing to the late spring at this station, some aecia were shedding spores at this time and others had not yet opened. Fortunately, this colony was readily accessible and a careful search showed that no other *Aecidium* occurred within fifty yards. Several infected plants were accordingly left for future observation in the hope that the rust would complete its life history. *H. dilatata* grows in very wet ground, often along streams or in shallow springs, although some of the hosts of *Aecidium Graebnerianum* occur in somewhat drier situations. The rusted colony was on a narrow strip of low, springy ground between a lake and a low cliff. *Carex* spp. formed about half the herbaceous cover and seemed to be the most likely alternate hosts.

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With the progress of the season, it became evident from observations on other species that the cool, short growing season permitted the survival of heteroecious Pucciniaceae (other than species, such as *Puccinia Poae-sudeticae*, that overwinter in the uredinial state) only if the alternate hosts are virtually contiguous. As time passed with no sign of infection on the sedges, it was feared that the telial host might have been adjacent only to plants that had been collected for the aecial specimen. Eventually, in mid August, a minute scrutiny of all the surrounding vegetation revealed a number of very small pustules, identifiable as such only under the hand lens, on a few plants of *Deschampsia atropurpurea*. Most of the pustules were on vegetative offsets, but a trace of infection occurred on one fruiting plant and allowed confirmation of the identity of the grass host. Only a few plants of *D. atropurpurea* were present and none were found elsewhere; nor was the *Aecidium* seen in any of many other colonies of *Habenaria dilatata*, *H. hyperborea*, or *H. obtusata* that were examined. The grass rust was heavy only on leaves within a few inches of *H. bracteata* and none occurred on plants three to five feet distant. The rust was left until 22 August, to allow further development of some of the immature sori, but was then collected for fear that all uredinia might be displaced by telia.

When microscopic examination was finally possible, the rust proved to be a species of *Puccinia* with teliospores possessing apical processes similar to those of *P. coronata*. In fact, the teliospores differed from those of *P. coronata* only in being distinctly shorter than the shortest forms of that variable species. The urediniospores also had appreciably thinner walls than is usual in *P. coronata*. The germ pores were obscure, partly, no doubt, because the walls were thin and only slightly pigmented. It is thus not known whether the number and disposition of the pores is different from that in *P. coronata* in which the pores are commonly about 6 to 8, scattered and often indistinct.

It must be emphasized that there is no possibility of this rust being *Puccinia coronata* in any accepted definition of that species. No *Rhamnus* or *Elaeagnus* occurs within many miles of the location. *Shepherdia canadensis* does occur, but it is restricted to the immediate coast and the nearest plants were some two miles dis-

tant. Furthermore, the correlation of the rust with proximity to the *Aecidium* was so striking as to preclude any doubt of the connection between the two stages.

An examination of the late W. P. Fraser's collections on *Deschampsia caespitosa* from Alberta and Saskatchewan, assigned by him to *Puccinia coronata*, showed that they are definitely that species; they have thicker urediniospore walls and much longer teliospores than the rust on *D. atropurpurea*. Barth. N. A. Ured. 431, on the same host from Wyoming, consists only of uredinia, but the spores are thick-walled and similar to those in Fraser's collections. These specimens apparently belong to some variety of *P. coronata* other than those recognized by Fraser and Ledingham (**2**).

A study of *Puccinia coronata* on one or more species each of *Agropyron, Agrostis, Avena, Beckmannia, Bromus, Calamogrostis, Festuca, Holcus, Lolium, Phleum, Poa* and *Scolochloa* gave a range of urediniospore wall thicknesses of 1.0–2.0 μ, except on *Agropyron* spp. in which it was 0.7–1.4 μ. The total range of teliospore lengths in these specimens was 33–78 μ with the exception of a collection on *Agropyron tenerum* in which the range was 30–70 μ. The measurements given below for the rust on *Deschampsia atropurpurea* show small but definite differences in these two characters, which, with the occurrence of the aecia on a monocotyledonous host, make it advisable to treat it as a distinct species. It is a pleasure to name this rust for Mr. I. L. Conners, of this Division, whose extensive knowledge of the graminicolous rusts has long been of great value to Canadian mycologists and plant pathologists.

Puccinia Connersii sp. nov.

Pycnia ignota. Aecia hypophylla, rarius epiphylla. Aeciosporae 16.0–23.0 × 12.5–19.0 μ; episporio hyalino, 0.5–1.2 μ crass., minute verrucoso. Celluli peridii 18–45 × 15–26 μ, pariete externo minute striato vel verrucoso, interno verrucis c. 3.0–3.5 μ alt. × 0.3–1.2 μ lat. obtecto. Uredinia et telia minuta, amphigena. Urediniosporae 17.5–21.5 × 15.5–20.5 μ; episporio pallide flavo, 0.5–1.2 μ crass., leniter echinulato, poris indetectis. Telia epidermide tecta, compacta. Teliosporae (23)25–36(39) × 13–20 μ, summae digitate appendiculatae; appendiculis 1.0–6.0 μ long., (0)3–4(8) in teliospora; episporio baso ochraceo 0.5–1.0 μ crass., summo castaneo 1.5–3.0 μ crass. appendiculis exclusis.

Aecia in foliis *Habenariae dilatatae*. Uredinia et telia in foliis *Deschampsiae atropurpureae*, prope Flumen Magnum Balaenarum, Canada.

Type. On *Deschampsia atropurpurea* (DAOM 23446), Great Whale River, Quebec, Canada, 22 Aug. 1949, in the Mycological Herbarium, Division of Botany and Plant Pathology, Ottawa, Canada; a portion of the collection in the Arthur Herbarium, Purdue University.

The type of *Aecidium Graebnerianum* has not been seen, but from material and descriptions available it is probably identical with *P. Connersii*.

There is no positive way of forecasting what other grasses will prove to be telial hosts for this species. If, as seems probable, all the collections ascribed to *Aecidium Graebnerianum* are the aecial stage of *P. Connersii*, there may be several grass hosts; but a consideration of the North American species of *Deschampsia* shows that they cover the range and habitats of the rusted orchids fairly completely, and there may be no other genus of grasses involved. *D. danthonioides* and *D. elongata* occur, according to Hitchcock (**3**), in open ground throughout the western range of *Ae. Graebnerianum*. *D. atropurpurea* occupies open or wooded, wet ground in the same region, across northern Canada, and southward on mountains in the east to New Hampshire; it does not occur at sea level in southeastern Quebec, but *D. caespitosa* var. *intercotidialis* is common along the shores of the Gulf of St. Lawrence in the vicinity of Rivière Ouelle and will probably prove to be the grass host for the rust in that region. *D. caespitosa* in its various forms covers most of the northern half of the continent and may be a host in some other localities; but, as already shown, the rust known on this species in Alberta, Saskatchewan, and Wyoming is *Puccinia coronata*. Although *P. Connersii* is probably not common in any part of its range, its apparent scarcity must be partly attributed to its inconspicuous aecia and telia.

An interesting feature of *P. Connersii*, and one that further supports its identity with *Ae. Graebnerianum*, is its apparent lack of pycnia. The immaturity of some of the groups of aecia in DAOM 23447 made this collection suitable for the study of pycnia, but no trace of any could be discerned.

ACKNOWLEDGMENTS

The writer wishes to acknowledge the advice of Dr. Geo. B. Cummins on the disposition and affinities of this species. His thanks are also due to Dr. R. C. Russell, who made available for study Prof. W. P. Fraser's collections of *Puccinia coronata* on *Deschampsia caespitosa* from the herbarium of the University of Saskatchewan.

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HISTOPLASMA AND BRAZILIAN BLASTOMYCES *

Eleanor Silver Dowding †

(with 6 figures)

Histoplasma capsulatum Darling is the causal organism of human histoplasmosis; *Blastomyces brasiliensis* (Splendore) Conant and Howell, of South American blastomycosis. *Histoplasma* is the more widely distributed, while *B. brasiliensis* is localized—chiefly in Brazil (fig. 1). There is no suggestion in the literature that they are closely related. They can, in fact, be distinguished one from the other in two ways.

First, in culture, the spores of *H. capsulatum* are more plentiful and are distinctively shaped, while those of *B. brasiliensis* are rarer and lack the distinctive irregular outline of *Histoplasma*.

Second, within the host cell, *Histoplasma* exists as a small oval cell 1.5 μ long, sometimes with a single bud. *B. brasiliensis* is a larger spherical cell 15–20 μ in diameter, surrounded by a number of buds.

However, in cultures of both fungi, spores may be lacking. Upon one occasion in this laboratory there were six cultures of a single strain of *H. capsulatum* on Sabouraud's medium. Two of them were one month old, two, three months, and the remaining two, four months, all growing vigorously. Only one of the six was producing spores. The other five possessed little to distinguish them from *B. brasiliensis.* In some cultures, the writer has seen microconidia of *H. capsulatum* in great abundance, but Howell (**9**), in his study, discovered so few that he considered them "obviously depauperate structures."

Again, Almeida studied the multiple-budding forms of *B. brasiliensis* as they occurred in animal tissue (**1**). He pointed out that

* This investigation was made possible by financial assistance from the National Research Council of Canada and the Dominion Department of Health.

† Mrs. E. S. Keeping.

the uppermost and lowermost slices of serial sections show groups of minute daughter cells separated from the parent cell. His photograph of these bodies within animal tissue strongly recalls *Histoplasma*. Only by searching in other sections would one find the larger cell to which they are attached. Then again, it has recently been shown that the spores of *Histoplasma* may reproduce by mul-

FIG. 1. Distribution of mycoses caused by *H. capsulatum* and *B. brasiliensis*. Adapted from Conant *et al.* "Manual of Clinical Mycology."

tiple budding (**8**). In short, the writer suggests that the two fungi are similar enough for medical mycologists, under certain conditions, to confuse them, and that a comparative study of their saprophytic phases would be in order.

Observations on *Histoplasma* have recently been published (**8**). Some of the results together with a discussion and interpretation

of them are given below, followed by a description of *B. brasiliensis* which confirms and extends Almeida's study (**1**).

MATERIALS AND METHODS

Six strains of *Histoplasma* and seven of *B. brasiliensis*, including *B. cerebriformis* were examined. Those studied in greatest detail were: *H. capsulatum* obtained from Dr. C. W. Emmons of the United States Public Health Service; *B. brasiliensis* from Dr. N. F. Conant of Duke University, and *B. brasiliensis* strain 630 from the National Collection of Type Cultures in Kew, England. A fungus sent for identification by Dr. K. B. Raper of the U. S. Dept. of Agriculture, was classified as *Sepedonium chrysospermum* Fr. and used for comparative study.

H. capsulatum was cultured on Sabouraud's medium, rice as prepared by Conant (**3**), Littman's agar, malt agar, dextrose broth, cysteine broth (**13**), and beef broth. *B. brasiliensis* was grown on Sabouraud's medium, plain agar, rice, cornmeal agar, malt agar, soil agar, soil, asparagin synthetic medium, and coffee agar (for the reason that South American blastomycosis is common among coffee workers). Of these substances, the most satisfactory for growth of *Histoplasma* was on Sabouraud's medium. *B. brasiliensis* grew well on Sabouraud's medium, plain agar, and rice, the best sporulation and most characteristic growth being on rice.

HISTOPLASMA CAPSULATUM

Histoplasma capsulatum grows at room temperature with a dense, white, fine, cottony, aerial mycelium sometimes bearing spores. The spores are of two kinds, microconidia and larger spores described in the literature as chlamydospores. The *microconidia*, which are borne laterally, are sessile, pyriform, frequently echinulate, and about 2.5 μ in diameter.

Since the *Histoplasma* "chlamydospore" is neither thick-walled nor resting, the term *macroconidium* is preferable for it, and will be used here. The macroconidia are about five times larger than the microconidia, spherical or pyriform, smooth or somewhat warty at first,[1] later, on Sabouraud's medium, becoming tuberculate with

[1] Mature macroconidia remain smooth on malt agar, Littman's agar, dextrose broth and cysteine broth.

radial projections, and taking on an amber color. A mucilaginous secretion exudes from the pits of these spores and envelops each of them.

The tuberculations of the macroconidia are characteristic and give the spores an appearance rather like *Chenopodium* pollen. Their shape, which may be obscured by the surrounding secretion, is pyriform, and their length about 2.5 μ. Howell (**9**) has pointed out that macroconidia of *Histoplasma* and the spores of *Sepedonium chrysospermum* both have finger-like projections. In the

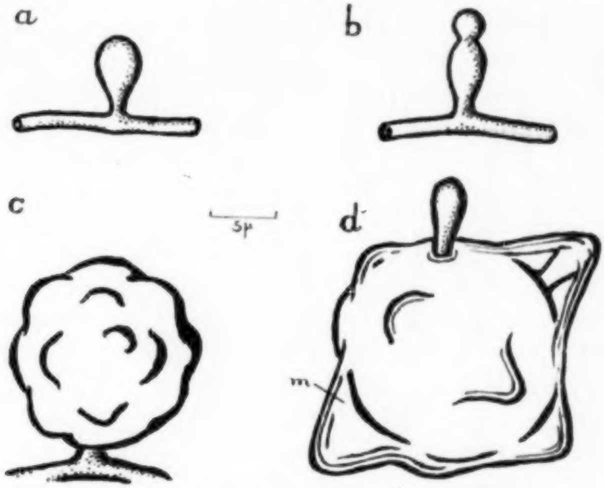

FIG. 2. The spores of *H. capsulatum*: *a* and *b*, microconidia; *c* and *d*, macroconidia; *a* and *c*, primary spores; *b* and *d*, secondary spores; *m*, mucilaginous secretion.

young spore of both they are hollow and contain protoplasm. However, the writer is convinced that they differ in that the *Sepedonium* spore-projections upon maturity become solid with wall-substance while those of *Histoplasma* remain hollow.

Morphological and cultural studies have shown that the conidial tuberculations are spores (**8**) (FIG. 6*c*). Because they are not readily detached, they are, like the microconidia of dermatophytes, aleurospores in Vuillemin's sense (**15**). They may give rise to numerous microconidia which become suspended in the drop exuded by the macroconidium.

We may classify the spores of *Histoplasma* as follows. A *primary* conidium is one that grows upon a hypha (FIG. 2*a* and *c*). A *secondary* conidium is one that grows upon a conidium (FIG. 2*b* and *d*). The diagrams in figure 2 are of microconidia, macroconidia, primary conidia and secondary conidia. The conidial tu-

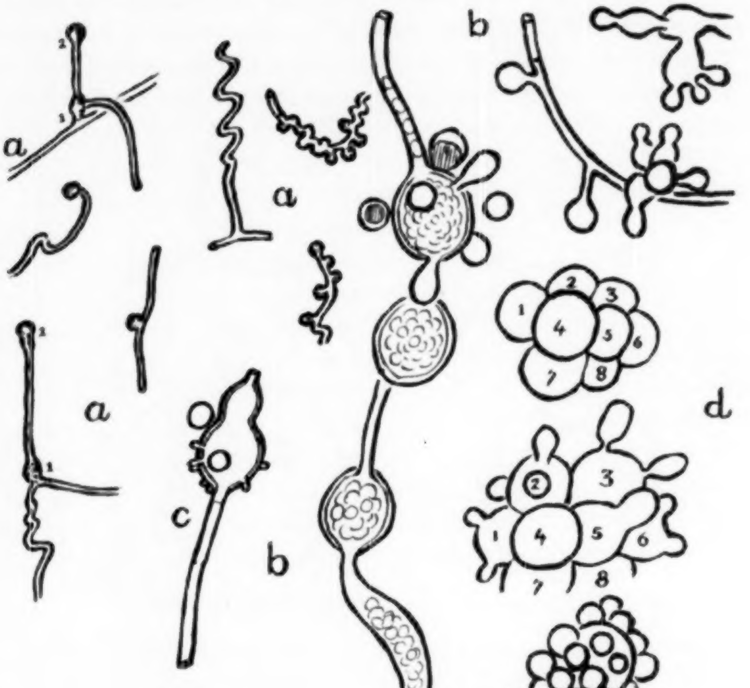

FIG. 3. The spores of *B. brasiliensis*: *a*, microconidia (shaded), *b–f*, macroconidia; *a* and *e* at 27° C; the rest at 37° C; *d*, growth during an interval of 24 hours. Further description in text. (× 1000.)

berculations (FIG. *2d*), according to this scheme, are secondary conidia.

Histoplasma grows as a yeast within the host, and also, under certain conditions (**13**), *in vitro.* Conant (**4**) describes the yeast colonies as small, white, moist, and pasty, resembling bacterial colonies. The yeast cell he describes as oval, 1.5–2 × 3 μ, reproducing by a single bud at the pointed end, at maturity enlarging and becoming spherical. Although the yeast cells usually bud singly, in this laboratory cells with as many as five buds have been photographed (**8**).

A previous paper (**8**) described the transformation of hyphae and conidia into yeast cells. In this transition, multiple budding is characteristic of the macroconidium. The secondary conidia which surround it enlarge, become spherical, detach themselves from the parent cell and act as yeast cells (FIG. *6b*).

BLASTOMYCES BRASILIENSIS

At room temperatures, *Blastomyces brasiliensis* is not vigorous; it grows slowly with a sparse, white, aerial mycelium. The center of young cultures may grow into the air as white tufts or bristles. On Sabouraud's medium cultures could often be identified by (1) their fine radial ridges and (2) the peculiar peripheral zone. This zone, instead of being white and fluffy like the rest, is often transparent and convoluted. Microscopical examination shows that the mycelium here is dead and disorganized. Almeida in describing cultures (**1**), mentions a "hard enveloping mass, a product of the hyphae."

Unlike *Histoplasma* with its tuberculate spores, *B. brasiliensis* on any of the media used, lacks conspicuous distinguishing morphology. The details of the mycelium are obscure. On agar the hyphae are narrow (less than 1 μ in diam.), delicate, indistinct, and tightly interwoven. Frequently lengths of cell wall become dissolved. The globular cell-contents may float in the medium and are then difficult to distinguish from spores (FIG. *3f*) or they remain as amorphous patches on the surface of the hyphae as described in *Neurospora* (**10**). The most conspicuous hyphae are certain branches that are wider and with denser contents than the rest, sometimes spiral, often hooked, and tending to fuse in pairs.

In general the mycelium suggests abnormal growth due to an unsuitable medium. A medium which furnished a more normal-appearing growth, although without spores, was synthetic asparagin broth with cotton-wool as a source of unsaturated fatty acid (**11**). The mycelium in this medium was submerged, coarse (with hyphae

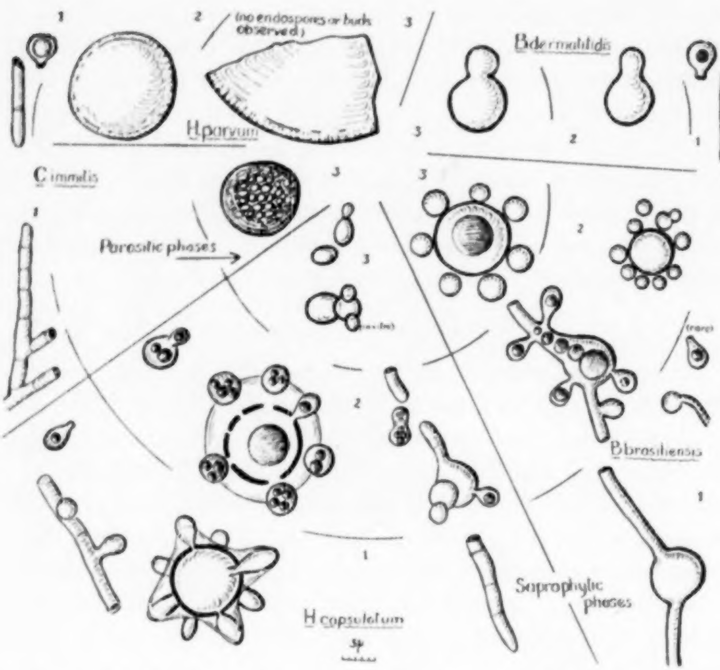

FIG. 4. Five systemic fungi: 1, at 27° C; 2, at 37° C; 3, in host. Reading anti-clockwise, the fungi are: *Haplosporangium parvum, Coccidioides immitis, Histoplasma capsulatum, Blastomyces brasiliensis* and *B. dermatitidis*. Only *C. immitis* has endospores.

2 or 3 μ in diam. and with dense contents), and lacking tangles or hyphal fusions.

The mycelium of *B. brasiliensis* has one feature that serves to distinguish it. It tends to grow in a three-dimensional network (FIG. 5*a*). Terminal swellings may produce radially several narrow hyphae which at their tips produce similar swellings (FIG. 3*a*, 1 and 2). The resulting mycelium resembles that of the parasite

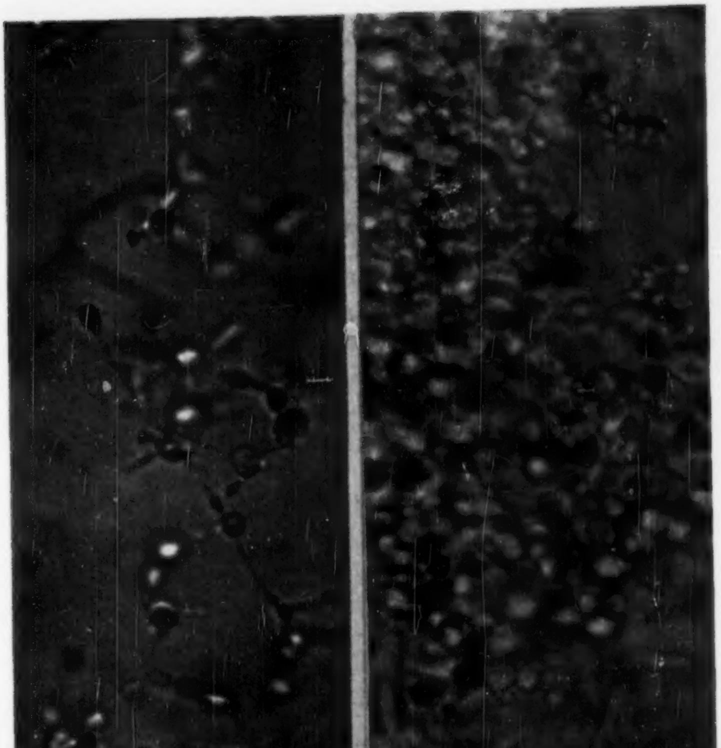

FIG. 5. *B. brasiliensis: a* and *b*, on rice at room temperature; *c*, on agar at 37° C; *a*, hyphal reticulum; *b*, microconidia; *c*, macroconidium. (× 1500.)

Syncephalis nodosa van Tieghem which envelops species of *Pilobolus* with a similar network and which is illustrated by Buller (**2**).

The conidia of *B. brasiliensis* have been described by Conant and Howell (**5**) as lateral, sessile, round to pyriform, 3–25 μ in diameter.

The writer will term the smaller spores, 1–4 μ in diameter, *microconidia*. They are terminal or intercalary. When terminal, a hypha may grow out from the spore near its point of attachment so that the spore is thrust aside and appears lateral (FIG. 3*a* center). Microconidia are scarce on agar, more numerous on rice. On rice they may be formed in clusters on spiral hyphae, so densely that their arrangement is difficult to make out (FIG. 5*b*). They are spherical and smooth, very seldom pyriform.

Cultures also produce larger terminal or intercalary cells, up to 25 μ in diameter (FIGS. 3*b*; 5*c*). Similar cells are formed in animal tissue. They have been described in the literature as *multiple-budding structures, yeast-forms, arthrospores,* or *chlamydospores*. The writer will term them *macroconidia*. They occur sometimes at room temperature, more readily at 37° C, and are distinguished by their ability to produce multiple buds, although by no means all macroconidia do so.

Compared to the macroconidia of *H. capsulatum,* those of *B. brasiliensis* are about twice the size, more variable in size and shape, and smoother contoured. As Almeida has observed (**1**), this smooth contour may be broken by small indentations.

At 37° C, *B. brasiliensis* grows as a multiple-budding yeast. Some strains, *e.g.* the English strain 360, are readily converted to the free-yeast form (FIG. 6*e* and *f*). In other strains, *e.g.* Almeida's *B. cerebreformis* now placed with *B. brasiliensis,* the budding cells are not readily detached, but tend to stay together. They grow as a pseudomycelium (FIG. 6*a*) or a tumor-like mass (FIG. 3*b*, upper right, and *d*). Such strains, growing at 37° C as microcultures, were observed successively under the microscope. At first a hypha possessed intercalary macroconidia at intervals along its length (FIG. 3*b*, center). Then the intervening parts swelled into spores, forming a bead-like chain (FIG. 6*a*). The members of the chain then grew by budding (FIG. 5*c*).

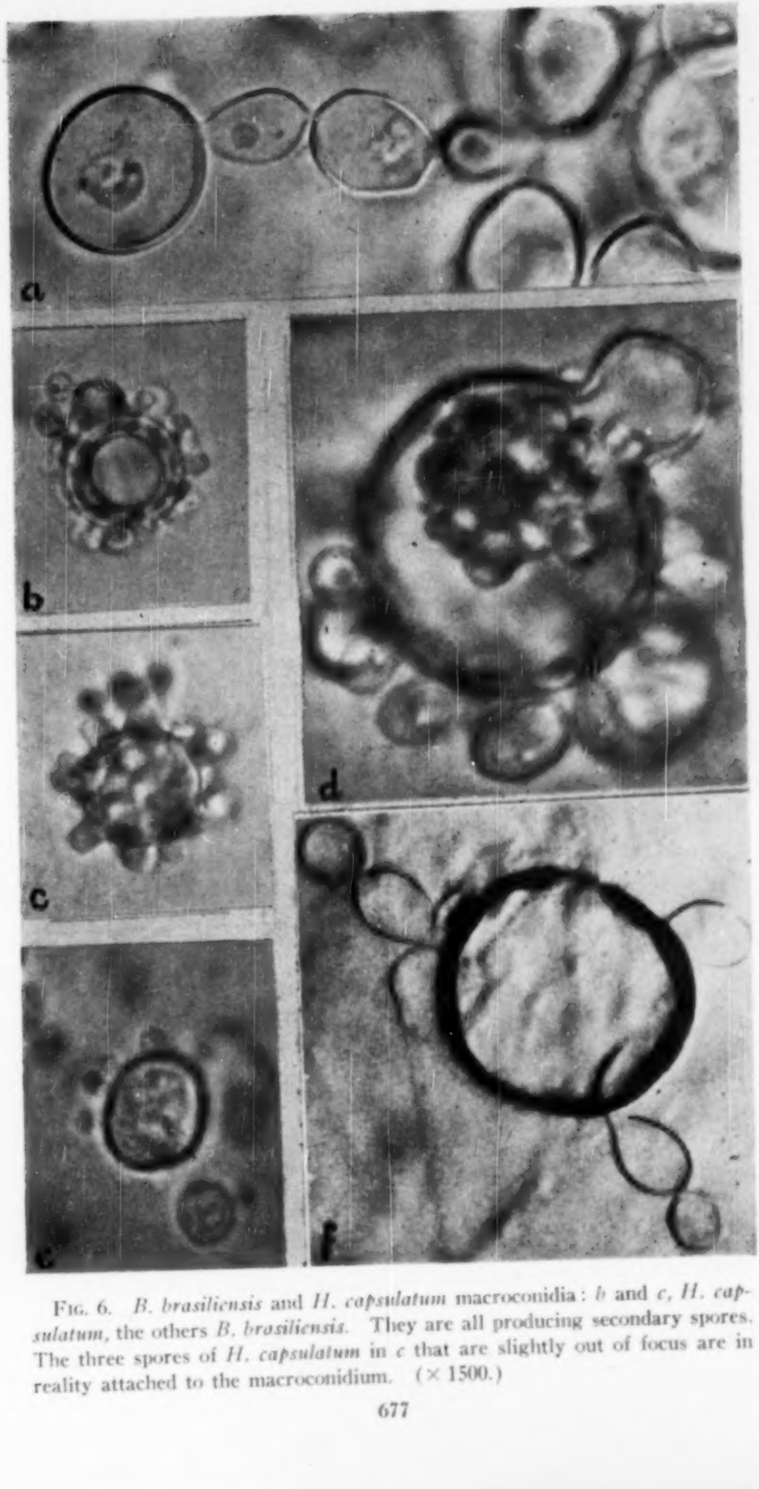

FIG. 6. *B. brasiliensis* and *H. capsulatum* macroconidia: *b* and *c*, *H. capsulatum*, the others *B. brasiliensis*. They are all producing secondary spores. The three spores of *H. capsulatum* in *c* that are slightly out of focus are in reality attached to the macroconidium. (× 1500.)

The multiple buds may be extremely small, even bacilliform (FIG. 3*c*). Sometimes they are difficult to see; for instance only careful examination of the third cell in figure 6*a* will reveal their presence.

DISCUSSION

It is well known that *Coccidioides immitis* reproduces within the host by the formation of *internal* spores, which are set free by the rupture of the spore wall. The ability to produce *external* buds separates the majority of systemic fungi from *C. immitis*. This difference between the species is shown (FIG. 4).

Of the fungi that reproduce by external budding, only *Blastomyces brasiliensis* is differentially diagnosed by its multiple buds in the animal tissue (FIG. 4). However, in the yeast phase of several other species, multiple budding has been observed.[2] Salvin found it occurring under certain cultural conditions in *Sporotrichum Schenckii* (**12**). De Monbreun (**7**) has photographed it in cultures from monkeys artificially inoculated with *Blastomyces dermatitidis*. The writer also has observed it in this species. In *Haplosporangium parvum*, figure 2*r* of a recent paper (**8**) suggests multiple budding.

The tuberculations in the macroconidia of *Histoplasma capsulatum* are interpreted as being external spores. These spores under appropriate conditions act as yeast cells. Multiple budding has, therefore, been demonstrated for an additional species.

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NOTES AND BRIEF ARTICLES

Chemical Activities of Fungi, by Jackson W. Foster. 648 pp. Academic Press, New York, 1949. $9.50.

There are two ways in which a book that covers a rather broad field of biology can be written. In one the author hurriedly assembles a hash of abstracts thickened with a few non-committal words of his own. The other way is for the author to consider carefully the observations reported in the literature of the subject using as a guide the hypothesis he thinks is most successful in unifying the material. This latter way, the one chosen by the author of this book, is the difficult one because knowledge of the subject is required; and much time and effort must be expended to produce the book.

An idea of the scope of the book can be obtained from the titles of the 19 chapters; Introduction, History, Perspective; The Methodology of Mold Metabolism; Chemical Nature of the Mold Mycelium; General Considerations of Mold Metabolism; Natural Variation; Mutations, Physiological Genetics, and Biochemical Syntheses; Trace Element Nutrition of Fungi; Lactic Acid Formation by Fungi; Alcoholic Fermentation by Molds; Oxalic Acid Metabolism; Fumaric and Other C_4-Dicarboxylic Acids; Citric Acid; Itaconic Acid; Kojic Acid; Gluconic and Other Sugar Acids; Carbohydrates Produced by Fungi; Nitrogen Metabolism of Fungi; Other Transformations in Fungi; and Microbiological Aspects of Penicillin.

The first seven chapters (46% of the pages) are devoted mainly to the physiology of the fungi considered in this book. The large amount of space given to the elements of physiology presumably reflects the author's opinion (shared by others) of the low state of current writing about fungous physiology.

In eight chapters (30% of the pages) are discussed acids, alcohols, and polysaccharides formed from carbohydrates. Products, and the possible mechanisms by which they are formed, are the main subjects of these chapters. The fungi are mostly a few spe-

cies of Rhizopus, Aspergillus, and Penicillium. The activities of yeasts, actinomycetes, and basidiomycetes are not considered.

Until we come to the chapter on penicillin, the contributions of American scientists to chemical activities of fungi are minor when compared with those of the Europeans and Japanese. Penicillin is the only antibiotic substance mentioned, as is proper, since it is the only one of importance formed by fungi. The commercial method for isolating penicillin as outlined in this book is of historical interest only, since the efficient plants use a much simpler process.

On nearly every page there is presented an unsolved problem, sometimes only by implication, so that this book can be used as a source for enough problems to occupy the interest of a generation of graduate students. Amazingly little is known about the details of many of the transformations discussed.

The index, as far as it goes, is excellent and is the kind all worthwhile books should have. Thirteen species of fungi are listed in the subject index. I hope that an organism index as good as the subject index is inserted when the book is reprinted. An organism index would make the work much more useful than it is to the beginning student for whom it was written. Names of fungi mis-spelled in the text should be corrected at least in the index.

Certain aspects of the book must be criticized. The title is more inclusive than the book. Skillful editing would have removed the verbiage and reduced the size of the book or released the space for other material. On many pages indentations apparently were inserted to break up the monotony of a solid page of type; they do not indicate the unity of thought that should constitute a paragraph. The shortcomings of the book are of minor importance. Nearly everyone who works with fungi will find something useful in it and will need to keep a copy handy.—F. W. KAVANAGH, NEW YORK BOTANICAL GARDEN.

MYCOLOGIA VOLUMES WANTED

Dr. Juan C. Lindquist, Curator of the Instituto de Botanica Spegazzini, La Plata, Argentina, writes that a fire has destroyed a portion of the Institute's library, although the collections themselves fortunately were not injured. Among other items destroyed were vol. 1, nos. 5 and 6, 1909; vols. 25 and 26, 1933–34; vol. 28, nos. 4 and 6, 1936; vol. 38, no. 1, 1946 and vol. 39, nos. 2 and 3, 1947. Funds are not available at present for purchases of books or periodicals and Dr. Lindquist hopes that anyone having duplicates or unneeded copies of the above volumes will be good enough to send them to the Institute.

MICROCARDS

Microcard copies of Fries's *Systema mycologicum* and *Elenchus Fungorum* have been prepared by the Microcard Foundation, Middletown, Connecticut. The reproduction is sharp and the text can be read with either a dissecting binocular or a hand-lens; Volume I occupies ten 3-by-5 cards. The four volumes are sold for $8.40; Volume I alone is $2.20.—A. H. SMITH.

CORRECTION

In "The Genus Gibellula on Spiders in North America," by E. B. Mains, *pleiopus* should read *leiopus* as used in the following names *Torrubiella arachnophila* var. *leiopus* (pp. 312, 314, 318 and 321), *Gibellula leiopus* (pp. 318 and 321), and *Gibellula arachnophila* f. *leiopus* (p. 318).

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